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NASA TT F-634

PROBLEMS OF SPACE BIOLOGY

Volume 11 - The Toxicology of Products of Vital Activity and Their Importance in the Formation of Artificial Atmospheres of Hermetically Sealed Chambers

by V. V. Kustov and L. A. Tiunov

"Nauka" Press, Moscow, 1969

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION • WASHINGTON, D. C. • MARCH 1971

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Translation of "Problemy Kosmicheskoy Biologii, Tom XI, Toksikologiya Produktov Zhiznedeyatel'nosti i Ikh Znacheniye v Formirovanii Iskusstvennoy Atmosfery Germetizirovannykh Pomeshcheniy," "Nauka" Press, Moscow, 1969

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

For sale by the National Technical Information Service, Springfield, Virginia 22151 — Price $3.00

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ANNOTATION

The toxicology of the products of vital activity and their significance in the generation of hermetically sealed living areas.

In this book we examine the history of the development of study concerning substances toxic to human beings and discuss questions relating to the formation of by-products of the vital activity of man in the course of normal matter exchange. Characteristics are given for the vital activity products which are released into the environment with exhaled air, with urine, excrement and those which are released by the sweat and oil glands of the skin. Study materials are explained relating to the toxic effects of basic metabolic discharges on animals and man. Principles are discussed for the hygienic standardization of the content of the products of vital activity in the atmosphere of hermetically sealed areas.

This publication is intended for a broad spectrum of physicians and biologists (physiologists, etc.).

PROBLEMS OF SPACE BIOLOGY, VOL. XI

THE TOXICOLOGY OF PRODUCTS OF VITAL ACTIVITY AND THEIR IMPORTANCE IN THE FORMATION OF ARTIFICIAL ATMOSPHERES OF HERMETICALLY SEALED CHAMBERS

V. V. Kustov and L. A. Tiunov

ABSTRACT: The formation of the gaseous products of vital activity is studied with an eye toward practical applications in the hermetically sealed environments of spacecraft on prolonged journeys, submarines, and the like. Data are drawn from a broad spectrum of literature references, describing analyses of feces, urine, sweat, expired air and intestinal gases, the formation of harmful substances by the processes of decomposition of these products of animal and human vital activity, and the effects of such products on experimental animals and humans. Formation of gaseous products of vital activity during normal metabolism is outlined in detail, as is the effect of certain environmental factors on the formation of gaseous metabolic products in the organism (such as acetone, ammonia, and carbon monoxide). The specific problem of composition and hydienic significance of the gaseous substances accumulated beneath t the clothing (or in a space suit) is discussed in detail. Chemicals and other means of treating urine, feces, etc., to retard formation of harmful or nauseating gaseous products are discussed and their relative merits compared. Principles of establishing the permissible limits of concentration (PLC) of such products of vital activity as carbon dioxide, carbon monoxide, hydrogen sulfide and the like in the atmospheres of hermetically sealed chambers are contrasted with methods of establishing the PLC for industrial working environments, and the inapplicability of the latter in the former situation is stressed. Thresholds are given for the toxic effects of such products on the organism.

INTRODUCTION

The scientific and technical progress that has been made, together with /5* rapid development of techniques of rocket construction and extensive development of studies of outer space, are making long-term manned space flights a reality. One of the problems that arises in the practical achievement of these splendid programs, which seemed fantastic just a short while ago, is the task

*Numbers in the margin indicate pagination in the foreign text.

of creating closed ecological systems. Forsaking the Earth for months and even years, a man in the hermetically sealed system of a spacecraft must create for himself an artificial atmosphere suitable for habitation under the difficult conditions of space flight. At the present time, maintenance of oxygen at the required level and removal of excess carbon dioxide and moisture from the atmosphere of hermetically sealed spacecraft intended for relatively short flights are accomplished faultlessly. Prolonged stays of human beings in hermetically sealed systems raise the problem of the removal of numerous chemical substances that contaminate such systems. In order to eliminate these substances, it is necessary to know their sources, the manner in which they are given off, the details of their effect on the organism, their safe concentration levels in the air and the effect of space-flight factors on the intensity of their production. The purpose of this book is to examine that part of the problem dealing with human vital activity. What toxic substances accumulate in a hermetically sealed system? What are the toxicological characteristics of these substances? How can their production be limited? What are the principles of their formation? The authors have attempted to answer these questions, which constitute one branch of the toxicology of ecologically closed systems, in order that their solution might aid in the achievement of reliable conditions of habitability in modern spacecraft intended for long-term flights.

CHAPTER I

HISTORY OF THE PROBLEM

The beginning of investigations of the gaseous products of human vital /7
activity can be traced to the 18th century, when oxygen and carbon dioxide were discovered and the negative effect on the organism produced by the carbon dioxide contained in expired air was observed (Priestley, 1772; Lavoisier, 1789, 1775; Scheele, 1777). Nearly a hundred years later, Pettenkofer (1862) suggested that expired air might contain organic compounds other than oxygen and carbon dioxide which would harm the air in poorly ventilated chambers. He proposed that carbon dioxide should be viewed as an integral (but not the sole) index characterizing the degree of pollution of the air in a chamber by the products of human vital activity. An extensive body of literature has been devoted to an investigation of these unidentified components of expired air. Particular stress should be placed on the series of articles by Brown-Sequard and d'Arsonval (1187, 1888, 1888a). These authors, who estimated the content of expired air from the degree of its biological effect, found that it contained highly toxic compounds. When a condensate of the expired air from dogs was injected subcutaneously into rabbits, it produced symptoms of intoxication and caused the death of some animals. The toxic effect of this condensate was also observed in experiments with guinea pigs and pigeons; it was administered intravenously, subcutaneously, intraperitoneally, orally and rectally. The authors suggested that this effect was produced by the presence of some unknown type of alkaloid in the expired air. At that time, it was known that expired air contains (besides carbon dioxide and oxygen) water vapor, methane (Tacke, 1884; Regnult, Reiset, 1849) and ammonia (Richardson, 1858). The toxic effects observed by Brown-Sequard and d'Arsonval could not be explained by the action of these known components of expired air. Therefore, these studies gave rise to a great many other investigations of the biological effects of the components /8
of expired air and stimulated a long discussion of anthropotoxins, kenotoxins and zootoxins (Zhandr, 1897; Wurtz, 1888; Merkel, 1892; Lehman, Jessen, 1890;

Formanek, 1900; Wolpert, 1905; Weichard, 1908, 1911; Inaba, 1911; Konrich, 1914; Korff-Petersen, 1914; Lehman, 1919). The disagreement among the authors was concerned mainly with the hygienic significance of the problem. The very fact of the presence in expired air of chemical substances besides nitrogen, oxygen and carbon dioxide did not arouse serious dispute. The list of such substances, besides the methane and ammonia mentioned above, was soon supplemented by carbon monoxide (Nicloux, 1898; Rathery et al., 1932), acetone (cf. Widmark, 1920; Hubbard, 1920) and a number of other organic substances oxidized by permanganate (Ransome, 1870). The greatest difficulty was encountered in separating the combination of these organic compounds in expired air. Due to the insufficient precision of existing methods of chemical analysis, these compounds were studied by using biological tests. A great many studies were devoted to explaining the nature of the toxic action of expired air on the isolated frog heart. Peters (1906) showed that a condensate of expired air inhibits the activity of the isolated frog heart. Subsequently, Lang (1914) was unable to substantiate these data. On the other hand, M. I. Gramenitskiy and I. I. Sivertsov (1935) and later Ye. N. Pavlovskiy (1937) again found that a condensate of expired air retarded the activity of the isolated frog heart. This research is of tremendous significance since it demonstrated the possibility of significant variations in the toxicity of expired air in individuals depending on their age and health. These data did not find applications in hygienic practice, however, because the effectiveness of the means of ventilation of residential and work areas was considered to be completely satisfactory as far as the carbon dioxide content was concerned. The accumulation of carbon dioxide and decrease of oxygen in the air was used to explain the death of persons in closed, unventilated rooms. This was the reason given, for example, to explain the death of 72 passengers aboard the steamer "Londonderry," who perished in their cabins when the latter were tightly battened down during a long storm (Morkotun, 1907).

The literature on the chemical composition of expired air places most emphasis on carbon dioxide and oxygen (Liljestrand, 1925). The initial experiments involving the hermetic sealing of individuals in small-volume systems

for uninterrupted periods of 6-8 hours showed that the duration of a harmless stay in a sealed chamber with no means of ventilation or regeneration of the air is determined by the concentration of CO_2 which does not affect working capacity. In this regard, the oppressive effect of the foul-smelling substances excreted by human beings was recognized (Erisman, 1887; Aver'yanov, Vladimirov, Grigor'yev, Kravchinskiy, Rylova and Smukhin, 1935). But it was CO_2, as the indirect index of the quality of the atmosphere, that served as the criterion for evaluating the ventilation in aircraft cabins (Spasskiy, 1940) and the regeneration of the air aboard submarines (German, 1943).

The design and construction of hermetically sealed systems, especially the cabins of modern aircraft, require an exact knowledge of the composition of the products of human vital activity that are liberated into the surrounding medium. The research of earlier authors, devoted to study of the organic components of expired air, took on new significance in correction with the design and construction of ecologically closed systems intended for long and continous occupation by human beings.

However, expired air is only one source of the products emitted by human vital activity. The excreation of products of vital activity in the perspiration, intestinal gases, urine and feces is of great importance (Tarkhanov, 1886; Korenchevskiy, 1909). Thus, urea, volatile fatty acids and other substances (cf. the reports by Levashova, 1895, and Zhandr, 1889, etc.) are excreted from the surface of the skin in perspiration. Ya. L. Okunevskiy also wrote about this in 1920, in discussing the problem of the composition of the air aboard submarines of the Russian navy.

The chemical composition of intestinal gases has also been studied in sufficient detail. It was shown in early investigations that the intestinal gases contain a much higher concentration of carbon dioxide and hydrogen and much lower concentrations of oxygen than atmospheric air (Ruge, 1862; Schierbeck, 1892; Boycott, Damant, 1907). The presence of significant quantities of methane in the intestinal gases was also observed (Fries, 1906).

These brief historical items indicate that attempts to determine the composition of the gaseous products of human vital activity have been in

progress for more than two centuries. These efforts have been completed at the present time with a certain degree of success. The use of the latest accomplishments in analytical chemistry, particularly gas chromatography and infrared spectrometry, have made it possible to determine to a significant degree the composition of the gaseous products of human vital activity and to evaluate their toxic properties and their importance to the formation of ecologically closed systems.

CHAPTER II

FORMATION OF GASEOUS PRODUCTS OF VITAL ACTIVITY DURING NORMAL METABOLISM

The study of the problem of the formation of gaseous products of vital /10
activity in the course of normal metabolism is concerned primarily with a determination of their chemical nature.

At the present time, more than 400 chemical compounds have been found to exist in metabolic products. They are classified as follows, depending on the excretory pathway: 149 substances enter the surrounding air with the expired air; 183 are excreted in the urine, 196 in the feces, and 271 from the surface of the skin (Weber, 1967). Of course, the toxicological significance of all of these products is not the same. S. G. Sharov, V. V. Kustov et al., (1966), Yu. G. Nefedov et al., (1967) and F. Kautroun (1967) listed the following compounds whose presence is important for the formation of atmospheres for closed systems: ammonia, phenols, methane, hydrogen, indole, skatole, amines, organic acids, carbon monoxide, acetone, mercaptans, hydrogen sulfide, as well as ethyl and methyl alcohol. All of these compounds belong to the so-called trace gaseous products of metabolism, in contrast to the carbon dioxide and water vapor that are the principal (and carefully studied) final products of human metabolism. In the present chapter, we shall consider the formation during vital activity of only the trace compounds: acetone, ammonia, amines, carbon monoxide, phenol, indole, skatole, carbonic acids and alcohols.

Acetone

Acetone is a normal product of metabolism, constantly formed in the process of metabolism in mammals (Schwarz and Rothkopf, 1936; Greenberg and Lester, 1944). Acetone is excreted primarily in the expired air and in the urine. It has also been shown that acetone excretion through the skin is possible. Approximately 10% of the total amount is excreted in this manner (Parmeggiani and Sassi, 1954). The intensity of acetone excretion depends on its level in the blood. There is a direct relationship between the levels

of acetone in the blood and in the expired air (Stewart and Boettner, 1964). /11
Brechner and Bethune (1965) confirmed this relationship in experiments using dogs.

Data on the acetone level in human blood and in the blood of various experimental animals are given in Table 1.

TABLE 1. ACETONE CONTENT IN HUMAN AND ANIMAL BLOOD

Type	Acetone, mg %	Source
Man	1.5	Marriott, 1914
Man	1.3 - 1.8	Sulimovskaya, 1945
Man	1.5	Makeyev, Gulevich and Broude, 1947
Man	0.3 - 2.0*	Nadeau, 1952
Man	2.0 - 2.5	S. Balakhovskiy and I. Balakhovskiy, 1953
Man	2.9 ± 0.3 g/ml (serum)	Levey, Balchum, Medrano, and Jung, 1964
Man	0.0 - 0.1	Asatiani, 1964
Dog	0.06	Vladimirov et al., 1945
Dog	2.2*	Grosman, 1951
Rabbit	1.84 ± 0.02*	Linyucheva, Tiunov, and Kolosova, 1963
White rat	1.59 ± 0.1*	
White mouse	1.25 ± 0.05*	
	1.84 ± 0.9*	

*Determination performed jointly with acetoacetic acid.

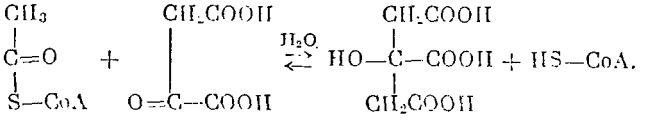
Acetone, together with acetoacetic and β-hydroxybutyric acids, constitutes the group of so-called ketonic bodies, whose level in the blood is determined by the course of the reaction involving metabolism of carbohydrates and fatty acids. The original product of the formation of ketonic bodies is active acetate, acetyl coenzyme A. The acetate fragment is formed by oxidation of fatty acids according to the general formula

$$R{-}CH_2{-}CH_2{-}COOH + 2H_2O \xrightarrow{+H} RCOOH + CH_3COOH. \quad (1)$$

Another source of acetyl coenzyme A is the conversion of carbohydrates to the pyruvate stage:

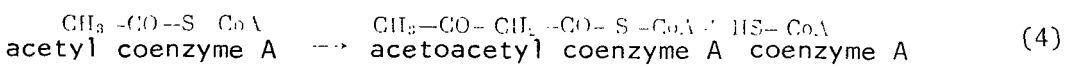
$$\text{Pyruvic acid} + NAD^+ + \text{coenzyme A} \rightarrow \text{acetyl coenzyme A} + CO_2 + NAD + H + H^+ \quad (2)$$

The reaction of the first stage of the Krebs citric acid cycle can also serve as a source of acetyl coenzyme A. In this stage, there is condensation of acetyl coenzyme A and oxalacetic acid with formation of citric acid and coenzyme A. This reaction, which is catalyzed by citrosynthetase (citro-oxaloaceto-lyase, 4.1.3.7) is reversible. With an excess of citric acid and a drop in the level of oxalacetic acid, the reaction shifts to form acetyl coenzyme A (Shtraub, 1965): /12



(3)

The majority of the acetyl coenzyme A is oxidized in the citric acid Krebs cycle to CO_2 and H_2O. Another portion of the acetyl coenzyme A is used in the numerous reactions of acetylization, and only a small portion of it goes into the formation of ketonic bodies. Acetyl coenzyme A is condensed to acetoacetyl coenzyme A. This reaction is catalyzed by the enzyme acetoacetyl coenzyme A--thiolase (acetyl-CoA: acetyl CoA--acetyl transferase, 2,3,1,9).



(4)

Acetoacetyl coenzyme A is used in the resynthesis of fatty acids and serves as a source for the formation of acetoacetic acid and cholesterol. Acetoacetic acid is formed by the deacylation of acetoacetyl coenzyme A. This reaction occurs in two stages (Linen, Helnning, Bublitz, Soerbo and Broeplin-Rueff, 1958). In the first stage there is condensation of acetoacetyl coenzyme A with acetyl CoA to form β-oxy-β-methylglutaryl CoA. In the second stage, the acetyl coenzyme A and acetoacetic acid split off from the β-oxy-β-methylglutaryl CoA. At the present time, data are available which indicate the possibility of direct hydrolysis of acetoacetyl coenzyme A to acetoacetic acid. An enzyme which catalyzes this reaction has been extracted from the liver (Drummond and Stern, 1960). This possibility was also indicated by Segal and Menon (1960) in their *in vitro* experiments with mitochondria of rat liver. Evidently both direct formation of acetoacetic acid from acetoacetyl

coenzyme A and indirect formation via the β-oxy-β-methylglutaryl CoA stage occur in the organism.

The level of acetoacetic acid in the serum of a healthy human being is 2.04 ± 0.6 mg% (Klein and Oklander, 1966). Acetone is formed by the decarboxylization of acetoacetic acid. Not all of the acetoacetic acid becomes acetone, however. A portion undergoes degradation to β-hydroxybutyric acid. The decarboxylization of acetoacetic acid to form acetone occurs spontaneously, but its conversion to β-hydroxybutyric acid is catalyzed by the enzyme 3-oxybutyrate dehydrogenase (D-3-oxybutyrate; NAD-oxide reductase).

The rate of oxidation of acetoacetic acid in man is 35 mg/kg/hr /13
(Leytes and Lapteva, 1967).

The formation of acetone during normal metabolism is shown in Figure 1. Formation of acetone and other ketonic bodies occurs in the liver (Van Stalie and Bergen, 1961). The content of ketonic bodies in the rat liver is 2.66 mg% according to data from O. N. Abbakumova-Zepalova (1950). V. S. Asatiani (1953) obtained similar figures, 15-33 μg/g of liver tissue. The ketonic bodies pass into the blood from the liver. Data on the content of ketonic bodies in the blood are presented in Table 2.

The ketonic bodies, like acetone, are formed in the liver and enter the blood. They are removed from the blood via the urine and expired air, and undergo partial conversion into other tissues. The ketonic bodies are not metabolized in the liver. This is indicated by the absence in the liver of the enzymes for their catabolism and the high level of ketonic bodies in the blood (Van Stalie and Bergen, 1961). Experiments with rats have shown that acetoacetic acid undergoes conversion in skeletal muscle (Beatty and Marco, 1960).

Acetone is distributed among the organs in the following order (in order of decreasing concentration): brain, spleen, liver, pancreas, kidneys, lungs, muscles, and heart Dervillee et al., 1949). The acetone content in the /14
tissues is much less than in the blood (Haggard, Greenberg, and Turner, 1944). For example, the acetone content in the blood is 4 times higher than in the heart (Kemal, 1937).

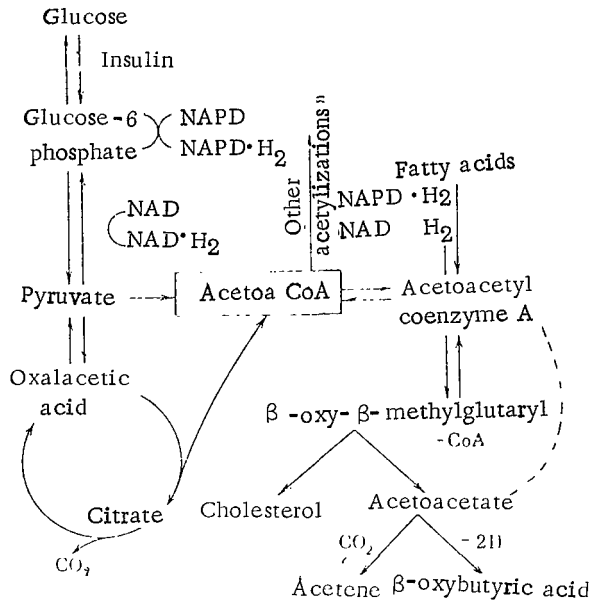


Figure 1. Formation of Acetone During Normal Metabolism.

TABLE 2. CONTENT OF KETONIC BODIES IN HUMAN AND ANIMAL BLOOD

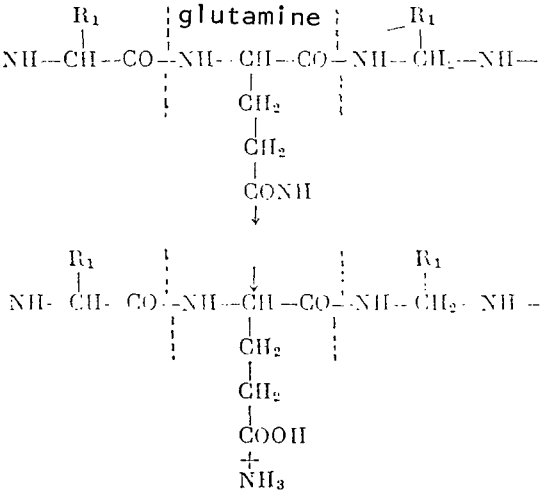
Type	Ketonic bodies, mg%	Source
Man	0.3 - 0.9 (plasma)	Weichselbaum, Somogvi, 1941
Man	0.2 - 0.8 (serum)	Stark, Somogvi, 1912
Man	9.23 - 15.31 (arterial blood)	Leytes, 1945
Man	10.82 - 11.90 (venous blood)	Leytes, 1945
Man	8.24	Leytes and Lirman, 1946
Man	6 - 9	Simonian and Daniel'-Bek, 1950
Man	4.6	Passmore, 1961
Man	0.71 ± 0.21 μM/l (serum)	Johnson, Sargent and Passmore, 1958
Dog	2.7 ± 0.9	Pushkina, 1963
Dog	8.6	Grosman, 1950
Rabbit	0.65 ± 0.35	Pushkina, 1963
Rabbit	1.0 - 7.7	Povolotskaya, 1940
Guinea Pig	17.5 ± 0.83	Linyucheva et al., 1968
White Rats	12.1 ± 0.20	Linyucheva et al., 1968
White Mice	11.80 ± 0.53	Linyucheva et al., 1968

The theoretical possibility of acetone conversion in the organism was mentioned as early as the 19th century (Schwarz, 1896). However, it is only in the last few decades, thanks to the use of tracer atoms, that reliable data have been gained on the conversion pathways of this metabolite in the organism. It was found in experiments using Wistar rats that about 50% of the acetone administered orally is oxidized to CO_2 (Price and Rittenberg, 1950). This pathway for the conversion of acetone may be imagined as follows: Acetone is carboxylized to acetoacetic acid (Koehler, Windsor and Hile, 1941; Plaut and Lardy, 1950), then converted to β-hydroxybutyric acid (Carpraro and Milla, 1950). The use of acetone labeled with hydrogen (deuterium) has made it possible to follow a radioactive label in cholesterin (Borek and Rittenberg, 1948). These data indicate that conversion of acetone occurs in the organism along the same metabolic pathways as its formation. In addition, the conversion of acetone forms three carbohydrate fragments of intermediate metabolism (Sakami and Lafayl, 1951). The radioactive carbon in acetone (C^{14}) occurs in pyruvate (Mourkides, Hobbs, and Koeppe, 1959) and then becomes carbonic acid through the reaction of the Krebs citric acid cycle. A certain part of the /15
acetone passes through the pyruvate stage to be converted into amino-acids: alanine, aspartic acid, glutamic acid, lactate, fatty acids (Lindsay and Brown, 1966), glucose (Ghiani and Nasta, 1947), and acetate (Zabin and Bloch, 1950).

Thus, acetone is formed in the organism in the course of normal vital activity, together with other ketonic bodies, by the conversion of fatty acids and carbohydrates. The original product of its formation is acetyl coenzyme A. A significant part of the acetyl coenzyme A is used in acetylization reactions, but a certain amount of it is converted into acetoacetyl coenzyme A and then into acetoacetic acid, and the product of the decarboxylization of the latter is acetone. The acetone formed in this manner is excreted in the expired air, in the urine and through the skin. A certain portion undergoes reverse conversion and is oxidized in the organism to CO_2.

Ammonia

This section is devoted to the processes leading to the formation of ammonia, the pathway by which it is converted, and the mechanisms by which it is excreted from the organism. Ammonia is one of the end products of nitrogen metabolism. The basic sources of its formation in the tissues[1] are the amino acids, amines, and amide groups of proteins. In muscle and brain tissue, the principal sources of ammonia are adenylic acid and glutamine (Embden, 1927; Vladimirova, 1938; Ferdman, 1950). Adenylic acid is deaminated by the formation of inosinic acid and ammonia. This reaction is catalyzed by the enzyme AMP-desaminase (AMP-aminohydrolase, 3.5.4.6). A number of studies have been devoted to the formation of ammonia from amide groups of proteins (Kane, 1957; Weil-Malherbe and Green, 1955). R. Vrba et al., (1954) state that the breakdown of amide functional groups of proteins in brain tissue form roughly one-fourth of all ammonia in the body. The following diagram has been suggested for this process:



(5) /16

[1] The formation of ammonia in the large intestine by the anaerobic splitting up of amino acids, as well as its formation in stored urine due to the splitting of urea under the influence of microbial flora, are discussed in separate sections.

The ammonia formed in this manner from protein glutamine reacts with the free glutamic acid to form glutamine. The formation of ammonia from the amide groups of proteins in the liver and nervous system may occur by another pathway as well. The biogenic amines (histamine, serotonin, noradrenaline) may interact with the amide groups of proteins. This forms a peptide chain and ammonia is excreted (Mycek and Waelsch, 1960). This reaction is catalyzed by the enzyme transglutaminase:

$$\text{protein}\text{—}CONH_2 + RNH_2 \xrightarrow{\text{transglutaminase}} \text{protein}\text{—}CONHR + NH_3. \qquad (6)$$

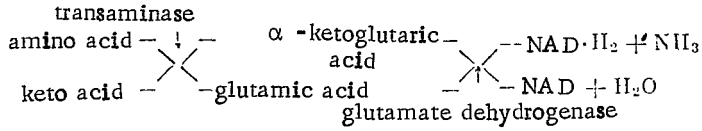
Oxidizing deamination plays an extremely important role in the process of protein formation. Aliphatic l-amino acids are subjected to deamination under the influence of dehydrogenase from l-amino acids (l-amino acid: NAD-oxidoreductase (deaminating, 1.4.1.5):

$$\underset{\text{amino acid}}{\overset{\begin{array}{c}R\\|\\H\text{—}C\text{—}NH_2\\|\\COOH\end{array}}{}} + NAD \xrightarrow{\text{dehydrogenase from } l\text{-amino acids}} \underset{\text{imino acid}}{\overset{\begin{array}{c}R\\|\\C{=}NH\\|\\COOH\end{array}}{}} + NAD\cdot H_2 \qquad (7)$$

$$\underset{\text{imino acid}}{\overset{\begin{array}{c}R\\|\\C{=}NH\\|\\COOH\end{array}}{}} \xrightarrow{\text{spontaneously}} \underset{\text{keto acid}}{\overset{\begin{array}{c}R\\|\\C{=}O\\|\\COOH\end{array}}{}} + NH$$

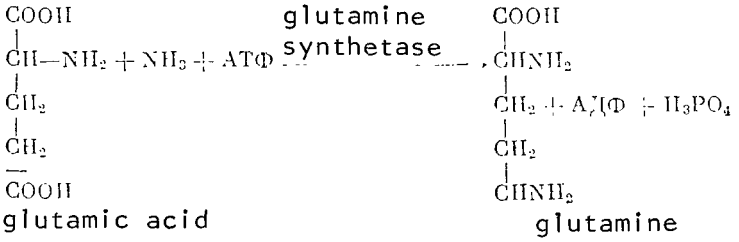
The glutamic acid-glutamine system is of great importance in the formation of ammonia by deamination of amino acids. As a matter of fact, the above-mentioned process of formation of ammonia by the oxidizing deamination of l-amino acids is limited to the liver and kidneys, where dehydrogenase from l-amino acids has been observed. The process of ammonia formation from l-amino acids following their transamination with the formation of glutamic acid is more widespread. Under the influence of the enzyme glutamate dehydrogenase [l-glutamate. NAD-oxidoreductase (deaminating) 1.4.1.2] the glutamic acid turns into ketoglutaric acid. Ammonia is liberated in the process. NADP may also act as a coenzyme with glutamate dehydrogenase, in addition to NAD. In this case the enzyme will be called glutamate dehydrogenase (NADP) /17
1.4.1.4. This enzyme is contained in the liver, kidneys, skeletal muscle, brain, heart, spleen, and thyroid gland (Arutyunyan, 1966). The diagram

showing the formation of ammonia in this case will have the following appearance (Rapoport, 1966):



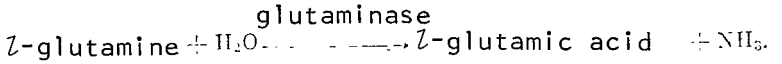
(8)

This system is reversible and can also serve for the liberation of free ammonia and the formation of amino acids from keto acids. Glutamic acid, in addition to oxidizing deamination, may also undergo other conversions. In particular, it may be acted on by the enzyme glutamine synthetase (l-glutamate: ammonia--ligase (ADP), 6.3.1.2). In the presence of ATP, it can react with ammonia to form diamine-glutamine.



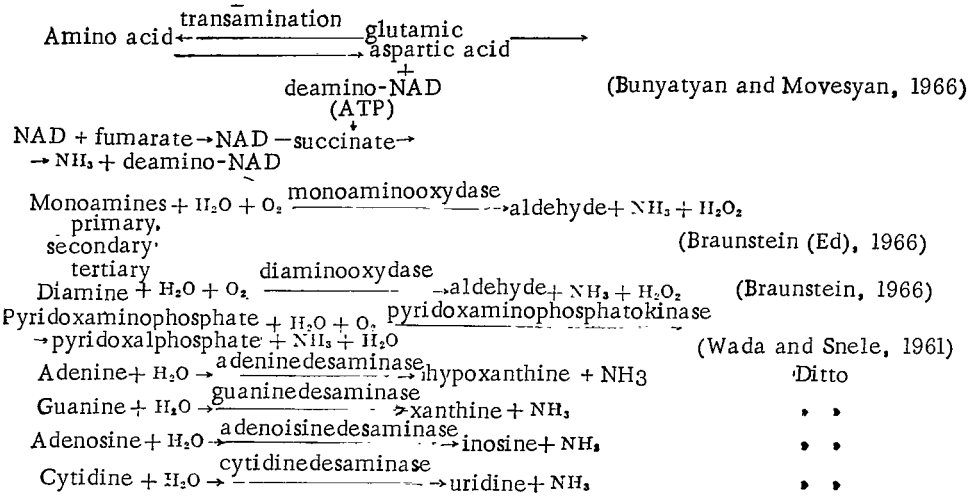
(9)

Glutamine as a component part of animal tissue was discovered by D. L. Ferdman (1941). This compound plays a very important role in the transport and rendering harmless of ammonia in various tissues (Ferdman, Frenkel' and Simakova, 1942). Under the influence of the enzyme glutaminase (l-glutamin-amidohydrolase, 3.5.1.2), glutamine is deamidated with the formation of ammonia:



(10)

In addition to the above processes which lead to the formation of ammonia, there are a number of other reactions in the organism which are related to the formation of ammonia but which apparently play a lesser role. They are as follows:



The nature of the processes of ammonia formation means that a certain /18
amount of ammonia is always to be found in the blood and tissues of the organism. Data on the ammonia content in human blood and in the blood of laboratory animals are given in Tables 3 and 4.

As we can see, the ammonia level in the blood and tissues is relatively low. This highly toxic metabolite does not accumulate in the organism, since the intense processes of ammonia formation are accompanied by reactions that destroy, utilize, or excrete it. In discussing the processes of des- /19
truction of ammonia in the organism, it is necessary to mention the work of Ivan Petrovich Pavlov, who showed that the breakdown of ammonia occurs in the liver during the formation of urea (Gan, Massen, Nentskiy and Pavlov, 1892). As of the present time, the process of urea formation has been studied in great detail. It is known as the Krebs-Henseleit cycle (Braunstein, 1949; Rapoport, 1966; Florkin, 1965).

TABLE 3. AMMONIA CONTENT IN HUMAN AND ANIMAL BLOOD

Type	Ammonia, mg%	Source
Man	0,004	Conway, Cook, 1939
»	0,05 (plasma)	Conway, Cook, 1939
»	0,01—0,036	Asatiani, 1953
	0,06—0.14	"
»	96,5±3,2 µg%	Pushkina, 1963
»	0,18 mg/100 ml	Asatiani, 1964
»	0,2—1,1 mg/100 ml (serum)	
»	25—30 γ/100 ml	Deuil, Godard, Gentilhomme, 1964
»	0,02—0,06	Mel'k, 1967
»	0,92±0,34 γ/ml	Muramatsu, Kejchiro, 1967
	0,96±0,26 γ/ml	
Dog	0,01—0,09	Makhlina, 1947
»	70,0±30,0	Pushkina, 1963
»	130—141 γ%	Zuidema, Sherman, Cullen, Kirsh, 1964
Cat	0,18—0,9	Winterstein, 1925
Rabbit	0,36	"
»	35,0±15,0	Pushkina, 1963
Guinea pig	90,0±20,0	"

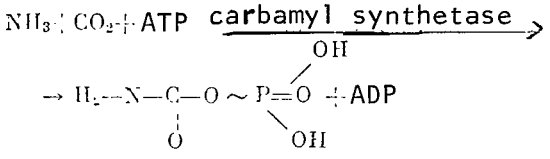
Commas indicate decimal points.

TABLE 4. AMMONIA CONTENT IN ANIMAL TISSUES

Type of animal and tissue	Ammonia, mg%	Source
Cat		
skeletal muscle	8,1	Silakova, 1954
cardiac muscle	3,6	
White rat		
brain	0,38 (0,28—0,45)	Vladimirova, 1957
brain	0,27 µM/g (0,08 - 0,4)	Gershenovich, Krichevskaya, and Bronovitskaya, 1957
brain	0,25	Richert, Darvson, 1948
White mice		
brain	0,49	Martinson and Tyakhepyl'd, 1957
skin (epidermal layer)	24,0	Roberts, Frankel, 1949

Commas indicate decimal points.

The first phase of urea formation is the reaction of free ammonia with carbon dioxide in the presence of ATP. This reaction is catalyzed by the enzyme carbamyl synthetase. As a result, carbamyl phosphate and ADP are formed:

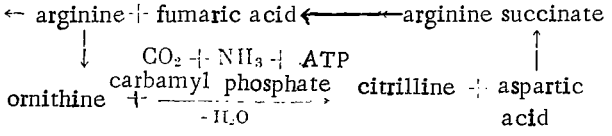


(11)

A certain amount of the carbamyl phosphate which is formed under the influence of the enzyme asparto-carbamoyl transferase (carbamoyl phosphate: l-asparto-carbamoyl transferase 2.1.3.2) is included in the synthesis of the pyrimidinic bases through the formation of carbamyl aspartic acid and uridine monophosphate. However, the majority of the carbamyl phosphate, under the influence of ornithine carbamoyl transferase (carbamoyl phosphate: l-ornithine carbamoyl transferase, 2.1.3.3) reacts with ornithine to produce citrulline and inorganic phosphorus. The use of carbamyl phosphate is regulated according to the feedback principle (Kachurina and Tiunov, 1966; Jones, Anderson and Hodes, 1961). /20

The next step in the synthesis of urea is the reaction of citrulline with aspartic acid and ATP to form arginine succinate. This reaction is catalyzed by arginine succinate synthetase (l-citrulline: l-aspartoligase (AMP) 6.3.4.5). The arginine succinate that is formed under the influence of arginine succinate lyase (l-arginine succinate arginine lyase 4.3.2.1) breaks down into arginine and fumaric acid. The last stage is the hydrolytic decomposition of arginine with the aid of the enzyme arginase (l-arginine ureohydrolase 3.5.3.1) to ornithine and urea. The ornithine again enters the reaction of the Krebs-Henseleit cycle while the urea is excreted with the urine and perspiration (Figure 2).

Hence, the bonding of ammonia in the Krebs-Henseleit cycle with the formation of urea occurs as follows: (Shcheklik, 1966):



(12)

The synthesis of urea occurs mainly in the liver. Now, however, data are available which indicate possibility of this synthesis in the brain as well (Bunyatyan and Davtyan, 1967; Gershenovich, et al., 1967; Bunyatyan, 1966).

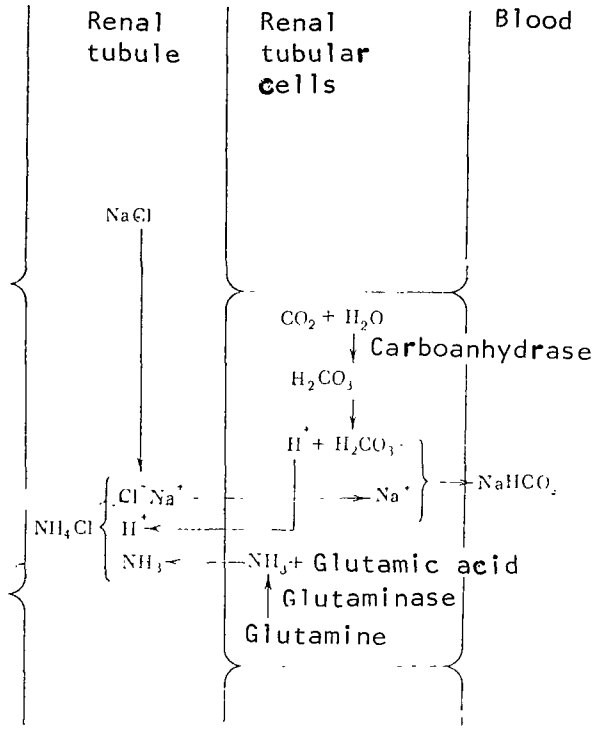


Figure 2. Diagram of Ammonia Excretion by the Kidneys.

Ammonia which is produced in the kidneys is excreted with the urine. Ammonia formation in the kidneys is achieved by the deamination of l-amino acids, especially glutamic acid and aspartic acid (Braunstein and Azarkh, 1944). The oxidase of l-amino acids functions in the kidneys; it was isolated by Blanchard and Green, 1944. The cells of the renal tubules actively secrete ammonia, which is formed as ammonia ions in the tubular fluid (Ulrich, 1960). This process is accompanied by the consumption of energy from adenosine triphosphate. The use of inhibitors of adenosine triphosphatase suppresses the secretion of ammonia in the kidneys (Bunyatyan, Oganesyan, Gevorkyan, 1967). The activity of carbonanhydrase (carbonate hydrolase 4.2.1.1) is

significant for the excretion of ammonia by the kidneys. This enzyme promotes the formation of H_2CO_3 from CO_2 and H_2O in the renal tubular cells. Carbonic acid is dissociated to form H^+ and HCO_3^- ions. Hydrogen is used to form ammonium chloride from ammonia. The chlorine ions for this process are formed by the dissociation of NaCl. The Na^+ ions react with the HCO_3^- ions to form $NaHCO_3$. Consequently, the higher the activity of the carboanhydrase, the more ions of H^+ and HCO_3^-. The more HCO_3^- ions there are, the more binding of Na^+ ions there is, which accelerates the dissociation of NaCl and leads to an increase in the number of Cl^- ions, which participate /21
(along with the hydrogen ions) in the formation of ammonium chloride from ammonia (Figure 2). The ammonia which is formed in the kidneys is not excreted completely with the urine. A certain portion of it enters the renal vein and thence passes into the general circulation (Gorzheyshi, 1967). A number of factors bear on the intensity of ammonia excretion by the kidneys. First of all, we should mention the role of the urine reaction. More ammonia is excreted in acid urine. The logarithm of the rate of ammonia excretion is inversely proportional to the pH of the urine (Jacina, 1961). A significant role is played by the activity of such enzymes as carboanhydrase, the oxidase l-amino acid glutaminase, as well as a number of their substrates (Propert, 1961).

Thus, the excretion of ammonia from the organism is determined by numerous reactions involving its formation, the intensity of urea formation and the functioning of the systems for its excretion in the kidneys. Disturbance of any link in these coordinated processes will lead to an increase in the ammonia level in the tissues and blood, increasing its excretion in the /22
expired air and perspiration and the development of pathological processes that produce its toxic effect.

Carbon Monoxide

For a long time the possibility of the formation of carbon monoxide in the human organism and in animals was subject to doubt. On the other hand, as early as 1894 a paper was published by Greant, which was later supported by Nicloux (1898, 1898a) and De-Saint-Martin (1898), on the presence of

carbon monoxide in dog blood. Later, carbon monoxide was found in the blood of healthy human beings (Nicloux, 1902, 1924, 1925) and in the expired air of dogs who had breathed an artificially prepared gas mixture that did not contain carbon monoxide (Rathery, Gley, Frane and Coursat, 1932). However, all of these investigations did not receive general recognition in view of the incompleteness of the analytical methods that existed at that time. Barth et al., (1953), noting this fact, denied the possibility of an endogenic formation of carbon monoxide in the course of normal metabolism. Peloquin (1951) pointed out that the presence of carbon monoxide in the blood of man and animals is the result of absorption of this poison from the surrounding atmosphere. Similar statements are to be found in the work of Truffert (1951). At the present time, however, thanks to the numerous studies that have been conducted with the use of diverse and highly sensitive methods, conclusive evidence has been obtained indicating that processes of carbon monoxide formation go on continuously in the organism. This problem is of tremendous importance in connection with the important role of the endogenic formation of carbon monoxide in the formation of the gas composition of the air medium of occupied hermetically sealed chambers (Bogatkov, Nefedov and Poletayev, 1961; Tiunov and Kustov, 1966). Surveys of the literature on the endogenic formation of carbon monoxide are found in papers by many authors (Tiunov, 1955; Tiunov and Kustov, 1966; Engstedt, 1957). Endogenic formation of carbon monoxide has been detected in man (Sjoestrand, 1949, 1949a), dogs (Coburn, Williams, Kahn, and Forster, 1964; Luomanmaeki, 1966), rabbits and guinea pigs (Kustov, and Ivanova, 1963; Metz and Sjoestrand, 1954), white rats (Kolosova, Tiunov and Kustov, 1969). In dogs, the rate of CO formation is 0.21 ± 0.09 ml/hr (Luomanmaeki, 1966). These findings were confirmed by Coburn et al., (1967), who obtained similar figures in their experiments (0.21 ± 0.05 ml/hr). According to our data, the endogenic formation of CO in white rats proceeds at an approximate rate of 0.026 ml/hr (Kolosova, Tiunov, and Kustov, 1969). In man, the rate of CO formation in the organism is 0.42 ± 0.07 ml/hr (Coburn, Blakemore, and Forster, 1963).

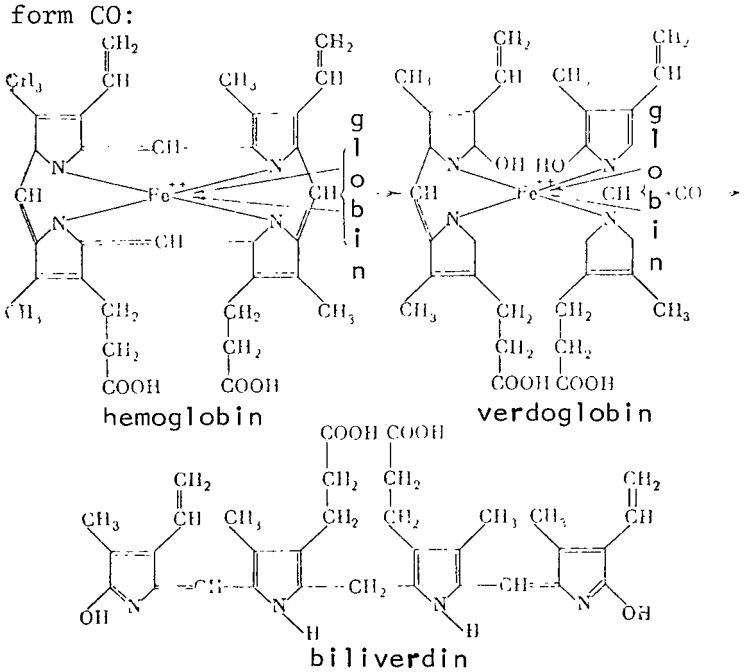
In the blood of male nonsmokers, the CO content is 0.38 ± 0.15%, while /23
for women it is 0.30 ± 0.11% (Hallberg, 1955). Due to the endogenic carbon

monoxide in the blood, there may be from 1.5% (Guillerm et al., 1953) to 3-4% carboxy-hemoglobin (Seifert, 1951; Petry, 1953).

There are a number of hypotheses regarding the mechanisms of endogenic formation of carbon monoxide. Some of them are only of historical interest at the present time. The greatest amount of attention has been attracted by a hypothesis which links the formation of CO in the organism to the degradation of hemoglobin molecules. It has been observed that the endogenic formation of carbon monoxide increases when there is increased decomposition of hemoglobin. In this case, if the catabolism of hemoglobin remains unchanged, the intensity of carbon monoxide formation will also remain constant (Sjoestrand, 1949a, 1950). When blood is incubated for 20 hours (+38°C), a certain amount of CO is formed. During hemolysis, the formation of carbon monoxide is increased. The intensity of the formation of carbon monoxide corresponds to the rate of degradation of hemoglobin (Sjoestrand, 1951, 1951a, 1952, 1952a, 1952b, 1953). In a number of diseases accompanied by hemolysis, an increase in the carbon monoxide level has been observed in the blood and expired air (Engstedt and Tretheim, 1953; Troell, Norlander, and Johonson, 1955). Sjoestrand (1951) suggested that the formation of carbon monoxide occurs in the oxidation of hydrogen in the α-methine cross link with subsequent breakage of the tetrapyrrol ring of hemoglobin. Ludwig, Blakemore and Drabkin (1957, 1957a), in experiments with the oxidation of crystalline hemin, showed that secretion of CO proceeds parallel to the formation of verdohemoglobin. These data were confirmed by Coburn et al., (1962, 1964), who used radioactive C^{14} in their studies. These authors injected glycine labeled with carbon (glycine-$2C^{14}$) into the reticulocytes. Thus, when hemoglobin was synthesized in the reticulocytes, the C^{14} entered the methine cross link of the tetrapyrrol ring. When such carbon-labeled hemoglobin was degraded, labeled carbon monoxide ($C^{14}O$) was formed. At the same time, the introduction of carbon-labeled formate does not lead to the occurrence of $C^{14}O$, which indicates that there is no relationship between the exchange of the monoxide remnants and the formation of carbon monoxide (Coburn, 1967). The radioactive label was observed in carbon monoxide when protoporphyrin and methemoglobin labeled with carbon were injected into the organism. In the latter case,

however, if the radioactive carbon was in the protein part of the hemoglobin, no radioactivity was observed in the carbon monoxide. This was still one more indication that carbon monoxide is formed in the process of normal metabolism and the degradation of hemoglobin due to the heme carbon (Coburn, Williams, White, and Kahn, 1967). The formation of carbon monoxide occurs in molar compounds with hemoglobin that is being destroyed (Coburn, Williams, and Forster, 1964; Coburn and Kane, 1965).

A certain amount of hemoglobin is still destroyed in the erythrocytes, /24
but most of it is degraded in the reticuloendothelial cells of the liver and spleen, which are the site of formation of endogenic carbon monoxide. This was shown in experiments on dogs performed by Wise and Drabkin (1965). Degradation of hemoglobin begins as a result of the oxidative interactions, the -CH= cross links between the I and II pyrrol rings of hemoglobin are replaced by -C(OH)= with simultaneous oxidation of Fe'' to Fe'''. In this manner, the hemoglobin changes to the green pigment verdoglobin (choleglobin) and the methine cross link is removed in the form of CO. Then the iron splits off the protein part, and the tetrapyrrol system acquires a linear form and forms biliverdin. The diagram below shows the method of breakdown of hemoglobin to form CO:



(13)

Diagram of Endogenic Formation of CO

The conversion of hemoglobin into verdoglobin is catalyzed by a special enzyme, oxidase, dependent on NADF-H_2. The maximum content of this enzyme is found in the liver (Nakajima, 1961).

Proceeding on the basis that the average lifetime of erythrocytes is 3 months, calculations were made to determine the rate of endogenic formation of carbon monoxide. It proved to be 0.3 ml/hr, and somewhat less than the /25
analytically determined value of 0.42 ml/hr, (Coburn, Blakemore, and Forster, 1963). These differences are explained by the fact that a portion of the endogenic carbon monoxide is formed by the degradation of other porphyritic compounds. Watson (1955) showed that about 20% of fecal stercoglobin is not of hemoglobinic origin. Engstedt (1957) states that carbon monoxide always accompanies the formation of bile pigments regardless of the nature of the original hemoproteids. Some of these other hemoproteids which (like hemoglobin) are sources of the formation of endogenic carbon monoxide are myoglobin, catalase, peroxidase, cytochromes and other porphyritic compounds.

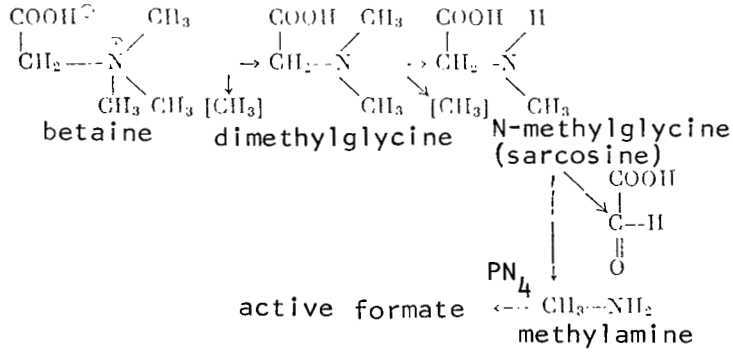
The process of excretion of endogenic carbon monoxide from the organism is discussed in the book by V. M. Karasik (1965). The carbon monoxide from the reticuloendothelial cells of the liver and spleen passes into the blood, where it is fixed by the hemoglobin and transported to the lungs. In the lungs, the carboxyhemoglobin is split off and the CO is excreted with the expired air. This natural process of removal of endogenic carbon monoxide is disturbed when the CO concentration in the surrounding atmosphere increases. Consequently, a strict condition for the removal of endogenic carbon monoxide from the organism is the absence or sufficiently low level of CO in the surrounding atmosphere.

Aliphatic Amines. Phenol, Indole, Skatole

Aliphatic amines are formed in the course of the reaction of intermediate metabolism. They are excreted from the organism with the urine, feces, and expired air. In 24 hours, a man excretes up to 10 mg nitrogen contained in the volatile amines (Rechenberger, 1940). More than 40 different amines are excreted with the urine; 26 of them have been identified, including methylamine, dimethylamine, ethylamine, ethanolamine, etc., (Perry and

Schroeder, 1963). A significant contribution to the total excretion of aliphatic amines from the organism is made by the processes of bacterial decarboxylization of the amino acids in the intestines. Such amines as methylamine, dimethylamine, diethylamine, propylamine, putrescine, cadaverine and others have been detected chromatographically in fresh feces (Rheenen, 1962). As an example of the endogenic formation of aliphatic amines in the process of metabolism, we can consider the reactions related to the formation of methylamine. This amine is a constant component of human urine. From 1.11-4.40 to 5.71 mg of methylamine nitrogen are excreted in the daily output of human urine (Iokhel'son, 1939; Natelson, 1947). The dog excretes from 0.69-1.09 to 1.87 mg of methylamine daily in its urine (Asatiani, 1953; Voynar, 1953).

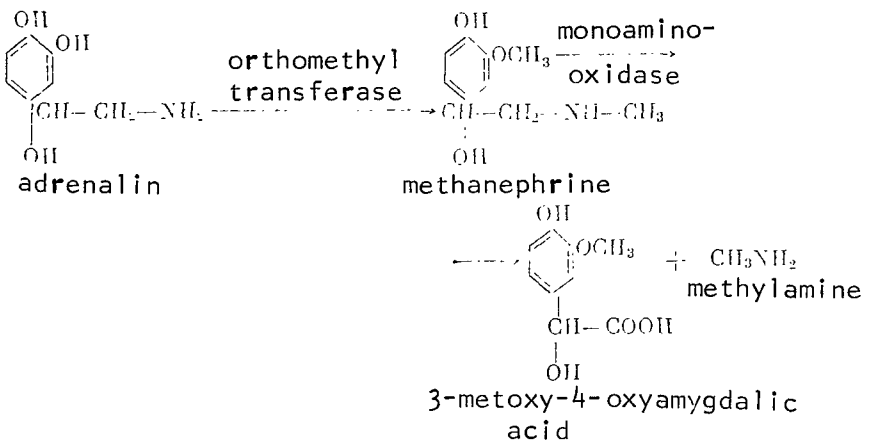
Methylamine is the intermediate link in the formation of active formate from methyl groups. The original product is betaine, formed by the oxidation of choline under the influence of the enzyme, choline dehydrogenase (choline: (acceptor) oxidoreductase). Through dimethylglycine, betaine is converted to N-methylglycine (sarcosine) which breaks up into glyoxalate and methylamine. Methylamine combines with tetrahydrofolic acid and is oxidized to the formyl group (Rapoport, 1966). The following is the diagram of the formation and conversion of methylamine:



(14)

Another aspect of metabolism in which methylamine is formed is the metabolism of adrenalin. Under the influence of the enzyme orthomethyl transferase in the presence of ions of manganese and S-adenosylmethionine, adrenalin is converted to methanephrine. Under the influence of monoamino-

oxidase (monoamine: oxygen-oxidoreductase-(deaminating)) this compound is converted to 3-methoxy-4-oxyamygdalic acid (Berzin, 1964). Then methylamine is formed as follows:

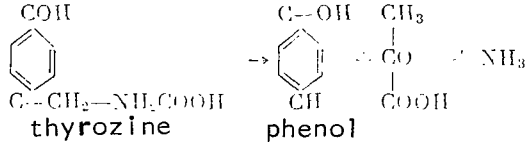


(15)

A number of aliphatic amines are formed in the organism by the initial decarboxylization of amino acids. Thus, the decarboxylization of cysteine is accompanied by the formation of cysteamine, which enters into the composition of coenzyme A,; propanolamine is formed in the decarboxylization of threonine; agmatine, putrescine and cadaverine are produced by the decarboxylization of arginine, ornithine, and lysine, respectively, and are observed in the composition of lysosome; decarboxylization of glutamine is accompanied by the formation of γ-aminobutyrate, which enters into the composition of brain tissue. Reactions of this type are catalyzed by the decarboxylases of amino acids, containing pyridoxal phosphate as the coenzyme. Thus, for example, the decarboxylization of l-arginine is catalyzed by arginine-decarboxylase (l-arginine-carboxylase, 4.1.1.19), the decarboxylization of ornithine, by ornithine decarboxylase (l-ornithine-carboxylase, 4.1.1.17); the decarboxylization of lysine, by lysine decarboxylase (l-lysine-carboxylase, 4.1.1.18) The amines that are formed are either excreted from the organism, or acetylized, or deaminized with the aid of monoaminooxidase (monoamines and diamines with a carbon chain containing more than 6 atoms of carbon) or diaminooxidase. /27

The formation of aliphatic amines in the organs and tissues of an organism during the decarboxylization of amino acids occurs within limited bounds. In the lumen of the intestine, however, thanks to the presence of highly active bacterial decarboxylases, these processes proceed very intensively. A number of amines are formed which are excreted primarily with the feces. These include putrescine, cadaverine, isoamylamine (a product of the decarboxylization of leucine), isobutylamine (a product of the decarboxylization of valine), and other amines.

Special attention should be given to the conversion in the intestine of such amino acids as thyrozine and tryptophan. Under the influence of bacterial enzymes in the intestine, thyrozine forms phenol, which is partly excreted with the feces and partly absorbed into the blood, so that it subsequently reaches the liver, where it undergoes breakdown in the conjugation reactions. The diagram showing the formation of phenol is as follows:



(16)

In the liver, phenol forms conjugate compounds with sulfuric or glucuronic acid. The phenylsulfuric or phenylglucuronic acid formed is excreted in the urine. A total of 80% of all the phenol contained in the organism is excreted in this manner (Deichmann, 1944). The remaining phenol is excreted in the urine, feces, and expired air. The enzymatic mechanism of the reactions of phenol conjugation is discussed in the paper by R. Williams (1965). The formation of phenylglucuronic acid occurs in several stages. The first stage consists in the reaction of glucoso-1-phosphate with /28 uridine triphosphate, forming uridine diphosphate glucose (UDP-glucose). In the second stage, UDP-glucose is oxidized to UDP-glucuronic acid under the influence of the enzyme UDFG-dehydrogenase (UDF-glucose: NAD-oxidoreductase 1.1.1.22). In the final stage, there is a transfer of the glucuronyl radical from the UDF-glucuronic acid. This reaction is catalyzed by UDF-glucuronyl transferase [UDF-glucuronate-glucuronyl transferase (nonspecific as to

acceptor) 2.4.1.17]. Another type of conjugation is the formation of phenylsulfuric acid. This conjugation also occurs in several stages. In the first stage, there is the formation of the activated form of the sulfate. Adenyl sulfate is formed by the reaaction of adenosine triphosphate (ATP) with the sulfate. This reaction is catalzed by the enzyme sulfatoadenylyl transferase (ADF: sulfate-adenylyl transferase 2.7.7.5). In the next stage the adenylyl sulfate reacts with a second molecule of ATF with the formation of 3'-phosphoadenylyl sulfate. This reaction is catalyzed by the enzyme adenylyl sulfate kinase (ATF: adenylyl sulfate-3'phosphotransferase 2.7.1.25). In the final stage, the sulfate radical is transferred from the 3'-phosphoadenylyl sulfate to the phenol, with the formation of phenylsulfuric acid. This reaction is promoted by the enzyme aryl-sulfotransferase (3'-phosphoadenylyl sulfate: phenolsulfotransferase 2.8.2.1.).

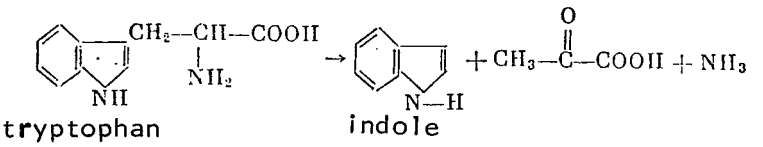
Despite the intensively proceeding conjugation reaction, free phenol is always present in the tissues and blood. Thus, in the rabbit brain the phenol content is 0.89 and in the liver, 0.65 mg% (Asatiani, 1953). In the body of the rat, 173 mg of phenol have been found (Deichmann and Oesper, 1940), while the phenol content in human blood varies from 16-42 (Teisinger et al., 1959) to 40-100 mg% (Gorzheyshi, 1967). The data on the content of free phenol in the human urine and in that of experimental animals are given in Table 5.

TABLE 5. FREE PHENOL CONTENT IN HUMAN AND ANIMAL URINE

Type	Phenol, mg/day	Source
Man	44,4	Travia et al., 1948
»	9,88—10,48	Schmidt, 1949
»	5—10	Porteous, Williams 1949
»	8,2	Teisinger et al., 1959
«	33,6	Asatiani, 1964
»	0,2—0,4	Gorzheyshi, 1967
Rabbit	9,7—12,7 mg/l	Porteous, Williams, 1949

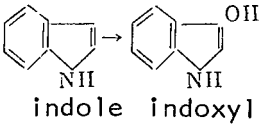
Commas indicate decimal points.

Indole is formed in the intestines as a result of the conversion of the amino acid, tryptophan. Under the influence of intestinal bacteria, this amino acid can be broken down by means of the tryptophanase reaction (Meister, 1961). Tryptophan is converted to indole, liberating pyruvic acid and ammonia: /29



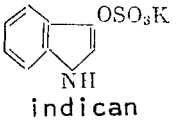
(17)

This reaction is catalyzed by an enzyme excreted by *E. coli* and containing pyridoxal phosphate as a coenzyme. Indole is partially excreted with the solid feces, giving the latter their unpleasant, specific odor, and is partially absorbed in the blood, reaching the liver where it is oxidized to indoxyl:



(18)

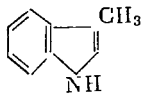
Indoxyl enters into the conjugation reaction with sulfuric or glucuronic acid and is excreted from the organism in the urine. The potassium salt of indoxyl-sulfuric acid has received the name of indican:



(19)

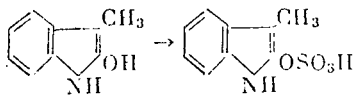
The daily urine output of an adult man contains from 1-38 mg (Pushkina, 1963) to 40-150 mg of indican (Gorzheyshi, 1967). In human blood, the indican level is 51 ± 29 μg% (Pushkina, 1963). According to other data the indican level in the blood varies from 0.03 to 0.16 mg% (Todorov, 1960).

The bacterial decomposition of tryptophan in the intestine is accompanied by the formation not only of indole, but also of a number of other products. In particular, the deamination of tryptophan and the partial breakdown of the side chain is accompanied by the formation of indole propionate, indole acetate, and methyl indole, or skatole:



(20)

Skatole, like indole, is partly excreted with the feces and partly absorbed in the blood, whence it passes to the liver. It is oxidized there to skatoxyl and enters into the reaction of sulfate conjugation. This results in the formation of skatoxyl-sulfuric acid, which is excreted from the organism in the urine: /30



skatoxyl skatoxylsulfuric acid

(21)

Alcohols and Organic Acids.

A number of authors have noted that certain amounts of ethyl alcohol are observed in the fluids and tissues of healthy individuals who have had no contact with this substance (Harger and Forey, 1963; Ward and Smith, 1965). These amounts are very small. According to Lester's data (1961), the ethanol level in the blood is 1.5 ml/l at most. Euecher and Redetzki (1951) state that the ethyl alcohol content in the human blood and organs does not exceed 0.2 mg%. Special studies using the chromatographic method have shown that the blood of healthy individuals who do not use alcohol has an ethyl alcohol content of less than 0.1 mg% (Walker and Curry, 1966).

Composite data on the ethyl alcohol content (in mg%) in the blood of individuals who do not use alcohol are given in the following: 0.0-0.027 (Harger and Joss, 1935); 0.2 (Buecher and Redetzki, 1951); 0.15 (Redetzki and Johannesmeier, 1956); 0.035-0.26 (Lundquist and Wolthers, 1958); 0.0-0.15 (Lester, 1962); 0.0002-0.234 (Eriksen and Julkarni, 1963); 0.1 (Walker and Curry, 1966).

Several authors have obtained still higher figures for the ethyl alcohol content in the blood of individuals who did not use alcohol. Thus, McManus, Contay and Olson (1966) reported that the content of normal ethanol in the human blood is 0.47-1.6 mg%. Certhoux and Ramet (1962) state that

when tranquilizers are given, the ethanol content of patients' blood reaches 60-100 mg%. Le Breton (1962), who performed similar studies, observed that of 25 subjects who received tranquilizers for a long period of time, 9 showed no ethanol in the blood while the figure did not exceed 10 mg% for the others. Breazeale (1965) found ethanol in the amount of 193 mg% in the blood of an accident victim who did not use alcohol. These data agree with the old studies by Leake, Swim and McCowlay (1940), who found that in rabbits with asphyxia the ethanol content in the blood increased to 150 mg%. However, a critical analysis of the studies on the ethanol content in the blood that gave high figures, conducted by Harger (1966) and Harger and Forney (1967) provides no basis for considering them reliable.

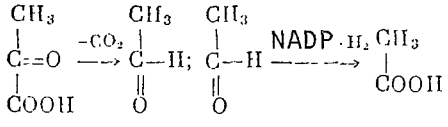
The overwhelming majority of the studies in various years and using /31
different methods have given figures on approximately the same level, no more than 0.26 mg%.

The endogenic ethanol present in the blood is excreted in the urine and expired air. A relationship has been shown to exist between the levels of ethyl alcohol in the blood and in the expired air. In healthy individuals who do not use alcohol, the ethanol content in the expired air is 0-2 mg/l (Freund and O'Hollaren, 1965). The figures given by other authors also fit into this range of concentration. Thus, Eriksen and Kulkarni (1963) found that the content of endogenic ethanol in the expired air of human subjects varies from 0.011 to 1.11 mg/l. Similar data are found in the literature for methyl alcohol. The presence of methyl alcohol in the blood of healthy individuals who had had no contact with it was noted by Western and Osborn (1949). Harger and Forney (1967) mention the presence of methanol in the blood of individuals in concentrations of 0.13-0.103 mg%.

The methanol concentration in expired air amounts to 0.06-0.49 mg% (Eriksen and Kulkarni, 1963). Leaf and Zatman (1952) state that due to the excretion of endogenic methanol from the organism, its content in human urine is 0.3 mg%. Eriksen and Kulkarni (1963), evaluating the problem of the possible sources of the formation of ethanol and methanol in the organism of healthy non-drinkers, find that these must include the use of fruits containing pectin, smoking, and the activity of intestinal bacteria.

Traces of methanol have been found in such fruits and vegetables as apples, oranges, carrots and celery (Western and Ozburn, 1949), while methyl alcohol may be formed in the enzymatic conversions in the intestines due to the metoxyl groups of pectin (Harger and Forney, 1967). It is also possible for methanol to be formed in the organism in other ways. In 1965, Axelrod and Daly reported that the pituitary glands of certain animals contain an enzyme which converts S-adenosyl methionine to methanol and S-adenosyl homocysteine. It is known that adenosyl methionine serves as the source of methyl groups, especially in the methylation of noradrenaline, ethanolamine, carnosine, and nicotinamide. In the case supporting the data of Axelrod and Daly, this source of methanol formation can be the determining level of methyl alcohol in the blood and expired air.

Bacteria and yeasts both contain the enzyme pyruvatodecarboxylase (carboxyliase 2-oxacid 4.1.1.1) which promotes the formation of acetaldehyde in the decarboxylization of pyruvic acid. Under the influence of reverse-acting alcohol dehydrogenase (alcohol: NADP-oxidoreductase 1.1.1.2) the acetaldehyde is reduced to methyl alcohol:



(22)

The possibility of the formation of ethanol in the intestines was pointed /32 out by Lundquist and Wolthers (1958), who suggested replacement of the term "endogenic ethanol" by the more precise term "enterogenic ethanol."

In human tissues, the conversion of pyruvic acid is accomplished by aerobic decarboxylization with participation of the enzyme system, pyruvatoxydase. This enzyme system evidently includes several individual enzymes (Shcheklik, 1926). The first stage of the conversion of pyruvic acid is catalyzed by pyruvatodehydrogenase (pyruvate: lipoate-oxidoreductase, 1.2.4.2). The pyruvic acid, reacting with oxidized lipothiamide, forms 6-S-acetylhydrolipoate. CO_2 is liberated in the process. Then the 6-S-acetyl-hydrolipothiamide reacts with coenzyme A. This reaction is catalyzed by lipoate-acetyl-transferase (acetyl CoA: dihydrolipoate-S-acetyltransferase

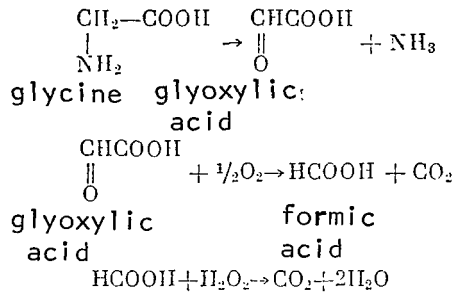
2.3.1.12). Acetyl coenzyme A and dihydrolipothiamide are formed as a result of the reaction. Then acetyl coenzyme A enters into the reaction of the Krebs citric acid cycle, while the dihydrolipothiamide reacts with NAD. This results in the formation of oxidized lipothiamide and $NAD \cdot H_2$. This reaction is catalyzed by lipoamide dehydrogenase (reduced NAD-lipoamide-oxidoreductase 1.6.4.3). Thus, the oxidizing decarboxylization of pyruvic acid in animal tissues forms active acetyl, and ethanol is not formed. Consequently, the principal source of "endogenic" ethanol is the bacterial processes in the intestines and the exogenic intake of this poison.

Human blood and tissues contain an extremely large amount of different organic acids. These include: formic acid, acetic acid, citric acid, isocitric acid, oxalacetic acid, α-ketoglutaric acid, succinic acid, fumaric acid, pyruvic acid, lactic acid, fatty acids, acetoacetic acid, β-oxybutyric acid, and others.

The blood of an adult individual contains 8-14 mg% of lactic acid, 0.5-1.0 (0.75) mg% of pyruvic acid, 80-100 mg% of citric acid, and 80-100 mg% of α-ketoglutaric acid. The fatty acid content is 275 mg% (250-300 mg%). Of this amount, about 18% consists of stearic acid, about 10% is palmitic acid, about 20% is oleic acid and 6% is linoleic acid. The higher saturated acids amount to about 3%. Unsaturated C_{20} acids amount to about 33%, while unsaturated C_{22} acids make up 10% (Todorov, 1960). Organic acids enter the organism with foodstuffs, are formed in the course of reactions of intermediate metabolism, or are synthesized by intestinal bacteria. They are converted in /33 the organism, and are oxidized to CO_2 and H_2O. Certain amounts are excreted into the surrounding medium with the expired air, perspiration, urine and feces[2]. For the sake of an example, let us examine the formation of formic acid. This acid is an intermediate product of the metabolism of glycine. In the liver, glycine is converted to glyoxylic acid, which is changed to formic acid. The formic acid is oxidized by endogenic hydrogen peroxide due to the peroxidasic action of catalase (hydrogen peroxide, hydrogen peroxide--oxido-

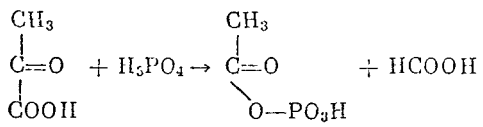
[2] See the corresponding sections (Chapters 4, 5 and 6).

reductase 1.11.1.5) to CO_2 and H_2O. A part of it is excreted from the organism in unchanged form. The diagram of the conversion of formic acid according to A. Meister (1961) is given below:



(23)

Formic acid is also formed in the intestines. Experiments with *E. coli* have shown that one pathway for the conversion of pyruvic acid among these bacteria is its reaction with phosphate. This forms acetyl phosphate and formic acid (Shtraub, 1965).



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Formic acid is partially excreted with the feces from the intestine, and partially absorbed into the blood, whence it enters into reactions of intermediate metabolism or is excreted in the urine, perspiration and expired air. The formic acid content in human blood reaches 0.4 mg% (Stepp and Zumbusch, 1920), while its content in the daily urine output varies from 15 to 18 mg (Kirkhgov, 1937). Altman and Dittmer (1964) present data indicating that the formic acid content in the urine is 0.8 (0.4-2.0) mg for each kilogram of weight. These same data are given in Spector's handbook (1956).

CHAPTER III

EFFECT OF CERTAIN ENVIRONMENTAL FACTORS ON THE FORMATION OF GASEOUS METABOLIC PRODUCTS IN THE ORGANISM

The excretion from the organism of the final products of vital activity /34 is subject to certain variations and depends on the influence of a number of factors in the environment. In this chapter, a number of examples will be used to illustrate the effect of the eating schedule, ionizing radiation, physical stress, hypoxia, hyperoxia and several other factors on the intensity of the formation and excretion from the organism of "trace" metabolic products.

Acetone

Schedule and Form of Nourishment. It is known that the acetone content of the urine increases during fasting or when the amount of carbohydrate in the diet is limited (Ferdman, 1966). Special studies of human subjects have shown that fasting causes a significant increase in the concentration of acetone in the expired air. Thus, in the case of fasting for 24 hours[3] the acetone concentration in the expired air increased from 0.97 ± 0.07 to 16.4 μg/liter. After fasting for 48 hours, the acetone concentration in the expired air rose to 32.6 μg/liter. At the same time, the acetone level in the urine rose from 0.8 ± 0.2 to 190 μg/liter (Levey, Balchum, Medrano and Hung, 1964). This increased acetone excretion from the organism is unstable. Two hours after taking food, the acetone concentration in the expired air falls to 6.80 μg/liter and returns to normal (0.85 μg/liter) in the days that follow. Increased acetone excretion is observed not only in complete fasting, but in partial fasting as well. Comparative studies show that ketonemia and ketonuria are present to a larger degree in persons who have a caloric intake of 1,000 calories than in individuals who have eaten 2,000 calories (Sargent, Johnson, Robbins, and Sawyer, 1958). What sort of mechanism increases acetone output by the organism during fasting? This question was studied in detail in the /35

[3] The drinking of water was not limited.

work of Forster (1967). During fasting, the stores of carbohydrates in the organism are used up rapidly. Thus, the glycogen content in rat liver falls from 42.1 ± 7.5 to 6.8 ± 1.9 mg/g of body weight after fasting for 12 hours. The principal energy source is the process of fat oxidation. The oxidation of fatty acids leads to an increase in the amount of acetyl coenzyme A in the organism. A lack of carbohydrates causes a halt in the formation of oxidized NADP in the glucose monophosphate shunt, required for resynthesis of fatty acids. A halt in the resynthesis of fatty acids in turn promotes the accumulation of acetyl coenzyme A. Thus, after 24 hours of fasting, the content of acetyl coenzyme A in the liver increases from 34.4 ± 2.8 to 70.5 ± 4.9 μM/g. Condensation of the accumulated acetyl coenzyme A leads to the formation of acetoacetyl coenzyme A and then acetoacetic acid. After fasting for 24 hours, the acetoacetic acid content in the blood increases from 1.3 ± 0.3 to 4.4 ± 0.7; after 48 hours of fasting it reaches 8.3 ± 1.4 mg% (Forster, 1967). Decarboxylization of acetoacetic acid leads to excess formation of acetone which is excreted with the urine and expired air..

Analogous phenomena are produced by incorrect ratios of carbohydrates and fats in the diet. Insufficiency of carbohydrates in the diet leads to increased excretion of acetone such as is observed in fasting (Azar and Bloom, 1963). A shortage of fat in the diet, however, causes an increase in ketonemia and ketonuria (Passmore, 1961). When living on a fat-rich diet, a man excretes 185.3 mg of ketonic bodies in the urine through conversion to acetone. At the same time, an excess of carbohydrates in the diet causes a drop in the excretion of ketonic bodies with the urine to 56.0 mg. With a mixed diet, the excretion of ketonic bodies with the urine in man is 72.3 mg (Oppel' and Rayko, 1940). Highly demonstrative experiments were conducted by Freund and Weinsier (1966) with cocoa butter extract, containing fatty acids with a carbon chain of 8, 10 or 12 carbon atoms (triglyceride). They found that injection of 30 ml of triglyceride into healthy subjects produced a sharp increase in the acetone content of the expired air. The maximum was recorded after 6 hours, when the acetone concentration reached 10 γ/l in the expired air. Increasing the dose of triglyceride to 100 ml increased the acetone content in the air to 26 γ/l. Normalization occurred 18 hours after

intake of fat. Addition of saccharose to the triglyceride reduced the ketogenic effect. The qualitative composition of the fat is important for acetone formation (Schoen, Lippach and Gelpke, 1959). Acetoacetic acid, *in vitro*, is formed more easily in the mitochondria of the liver from fatty acids with a short C_6 - C_{10} chain than from fatty acids with a long C_{16} - C_{18} chain (Kennedy and Lehninger, 1950). The ketogenic effect of fat is retained even in adrenalectomized and hypophysectomized animals (Mayes, 1962). The close /36
relationship of ketogenesis to lipo- and glucogenesis (Wieland, 1967) requires accurate balancing of the food ration.

Ionizing Radiation. According to the data in the literature the formation of ketonic bodies under the influence of X-rays is increased in the terminal stages of radiation sickness in white rats and rabbits (Ivanov, Zhulanova and Romantsev, 1954; Romantsev, 1956). At the same time, O. V. Popov, V. I. Nesterov and G. G. Ivanenko (1958), who used dogs, did not observe any significant changes in the level of ketonic bodies in the blood following irradiation. In view of the existence of contradictory data, we conducted joint experiments with L. A. Linyucheva (1968) including a special study to deal with the question of how X-rays affect the levels of acetone, acetoacetic and β-hydroxybutyric acid in the blood and urine of white rats. The studies were conducted using male white rats weighing 180-200 grams. The animals were exposed to X-rays in a RUM-3 machine. The irradiation conditions were as follows: Voltage = 180 kV, current = 20 mA, filters = aluminum (1.0 mm) and copper (0.5 mm); the distance from skin to focus was 60 cm; total dose = 700 r; dose intensity = 45.4 r/min. At various intervals following irradiation, the amounts of acetone, acetoacetic and β-hydroxybutyric acid in the blood and urine of the white rats were measured. The blood tests were made after 3 hours and after 6 days, while the urine was analyzed 24 hours and 5 days after irradiation. The test results are shown in Table 6.

TABLE 6. EFFECT OF X-RAYS (700 r) ON THE CONTENT OF ACETONE, ACETOACETIC AND β-OXYBUTYRIC ACID IN THE URINE AND BLOOD OF WHITE RATS.

Substance	Blood				
	Before irradiation	After 3 hours	P	After 6 days	P
Acetone and acetoacetic acid, mg%	1.20 ± 0.03	1.32 ± 0.03	0.015	1.36	0.003
β-hydroxybutyric acid, mg%	10.71 ± 0.3	10.05 ± 0.17	—	10.43 ± 0.26	—
Substance	Urine				
	Before irradiation	After 24 hours	P	After 5 days	P
Acetone and acetoacetic acid, mg%	2.10 ± 0.08	2.05 ± 0.08	—	4.31 ± 0.14	<0.001
β-hydroxybutyric acid, mg%	28.53 ± 0.7	29.47 ± 2.19	—	39.62 ± 2.01	<0.001

As indicated by the Table, the blood of white rats shows a small but statistically reliable increase in the content of acetone and acetoacetic acid even during the first hours following irradiation; this condition persists during the days which follow. On the 5th day following irradiation, the urine of white rats shows a sharp increase in the content of acetone, acetoacetic acid and β-hydroxybutyric acid. These results can be explained by the studies of Dubois, Cohran and Doll (1957) who showed that irradiation retards the oxidation of carbohydrates in the Krebs citric acid cycle. This /37 leads to an increase in the content of acetyl coenzyme A in the irradiated organism (Kuzin, 1962). The increase in the level of acetyl coenzyme A is promoted by partial fasting, characteristic of radiation sickness. An excess of acetyl coenzyme A will produce increased formation of ketonic bodies, as indicated in the preceding section. In addition, it must be kept in mind that during the development of radiation sickness the disruption of hormonal regulation has a definite effect on the process of ketogenesis. It is known that injection of cortisone into animals raises the level of ketonic bodies

(Vakaki and Yasuo, 1963). In radiation sickness, hyperfunction of the adrenal cortex is noted quite soon after irradiation, and is accompanied by a rise in the corticoid content in the blood (Van Couwenberge, Fischer, Vliers and Bacq, 1957). This fact obviously also plays a specific role in the development of acetonemia and acetonuria in white rats following irradiation by X-rays.

Other Factors. There are a great many factors that exert an influence on the development of ketosis. They include diseases or functional impairments of the activity of the gastrointestinal tract (constipation, for example), disturbance of the metabolism of fats and carbohydrates, etc. The intensity of acetone excretion depends on sex and age. Hormones have a certain effect on this process. Insulin has an antiketogenic effect, while the growth hormone from the pituitary increases ketogenesis (Passmore, 1961). Along with the hormonal effects, there is also the problem of the relationship between stress and ketogenesis (Sargent and Consolazio, 1951). Temperature increase reduces ketonemia and ketonuria (Sargent, Johnson, Robbins and Sawyer, 1958).

Water balance in the organism has a certain amount of significance for the formation and excretion of ketonic bodies (Passmore and Johnson, 1958).

The water balance in the organism is important to some degree for the formation and excretion of ketonic bodies in the organism (Chetverikova, 1962, 1962a). It has been found that under conditions of severe hypoxia the content of ketonic bodies in the liver increases by a factor of 2.6 (Chetverikova and Petukhova, 1962). In this regard, a certain amount of interest has been attracted by the statement found in the paper by V. S. Asatiani (1964). /38
The content of acetoacetic acid in micrograms/100 ml of urine was 60-110 in men and 85-95 in women. After a 25-day sojurn at an altitude of 1,200 m, the content of acetoacetic acid increased in men to 90-145 and in women to 110-120 μg%.

Ammonia

Ionizing Radiation. The information in the literature on the effect of ionizing radiation on ammonia excretion from the organism are contradictory. According to the data of Lamerton, Elson and Christensen (1953), radiation does not produce any qualitative or quantitative changes in the excretion of nitrogen, ammonia, urea, or amino acids. On the other hand, A. Yugenburg (1926) noted a drop in the nitrogen excreted with the urine following general irradiation. Similar data in experiments with rats irradiated with X-rays in the amount of 800 r were obtained by Jehote (1954), who found that the excretion of nitrous fractions in the urine decreased until the 5th day following irradiation. S. S. Perepelkin, on the other hand, in experiments with dogs which had been irradiated twice by a 400 r dose of X-rays (800 r in all) observed increased ammonia excretion in the urine. Increased ammonia excretion in the urine of white rats following irradiation with X-rays was described by Kolousek, Siracek, Zicha and Dienstbier (1965). T. N. Protasova (1958), in experiments with rats irradiated by X-rays in a dose that caused the death of some of the animals, found definite variations in ammonia excretion. It was only when a dose of X-rays that caused the death of all the animals was given that an increase of the ammonia level in the urine was observed.

The contradictory nature of the data in the literature is evidently explained by the fact that the various authors used different animals, carried out their tests over varying periods of time, and administered various doses of X-rays. In addition, they investigated only the ammonia content in the urine, which does not provide a sufficient basis for drawing conclusions regarding its total liberation from the organism into the surrounding atmosphere. In this regard, T. S. Kolosova undertook a special study of white rats. The problem consisted in explaining the question of how X-rays affect the total excretion of ammonia from the organism. The tests used male white rats weighing 180-200 g. Twenty animals were placed in a hermetically sealed chamber with a volume of 0.24 m^3. The concentrations of oxygen and carbon dioxide in the chamber were maintained automatically at

a constant level. The temperature in the chamber was 18-20°. The ammonia in the air of the chamber was determined daily. The ammonia level in the air of the chamber increased gradually. After 4 days, the amount of ammonia excreted by the rats reached 130 mg/m^3. Under similar conditions, white rats /39 that had been irradiated with X-rays at a dose of 700 r and then placed in a chamber on the 5th day after irradiation, excreted ammonia in significantly smaller quantities. When such irradiated rats were placed in a hermetically sealed chamber, the ammonia concentration at the end of 4 days reached only 69 mg/m^3. (Table 7 and Figure 3).

TABLE 7. EFFECT OF X-RADIATION (700 r) ON THE INTENSITY OF AMMONIA EXCRETION IN WHITE RATS.

Duration of stay in hermetically sealed chamber, in days	Ammonia in the air of the chamber, mg/m^3		Duration of stay in hermetically sealed chamber, in days	Ammonia in the air of the chamber, mg/m^3	
	intact	5-8 days after irradiation		intact	5-8 days after irradiation
1	11.0	9.8	3	117.0	54.0
2	82.0	32.0	4	130.0	69.0

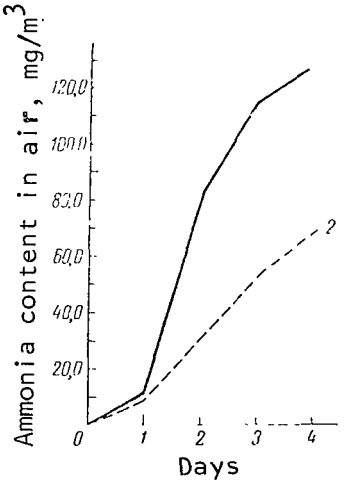

Figure 3. Excretion of Ammonia by White Rats Following Exposure to X-rays in a Dose of 700 r. Control (1) and Experimental (2) Animals.

As the Table and Figure show, the excretion of ammonia into the surrounding atmosphere was considerably less in the case of the irradiated animals than it was for the controls. The ammonia concentration in the air of the chamber in this case was determined by a number of factors: the intensity of excretion of ammonia by the animals in the expired air, urine and feces; the intensity of the bacterial processes in the urine and feces, accompanied by the formation of ammonia; the intensity of the change

of ammonia from the air in the chamber to the condensate. The latter three factors depend on the conditions of the external medium, which were exactly the same in the experiments with the intact and irradiated animals. Consequently, we may conclude that the differences in the ammonia content in the air of the hermetically sealed chamber are related to differences in the intensity of its excretion by the irradiated and control animals. These differences may be related to the fact that the irradiated animals show inhibition of the processes of deamination of amino acids. (Protasova, 1958), which constitute one of the sources of ammonia formation in the organism. Apparently there are also other mechanisms in this phenomenon that require special investigation. /40

Physical Stress. Physical stress causes an increase in the formation and excretion of ammonia. Thus, the ammonia content in the skeletal muscle of a cat at rest is 8.1 mg%, while under physical stress it is 11.8 mg%. In the cardiac muscle, the ammonia content at rest is 3.6 mg% and 5.4 mg% during work.

The increase in ammonia formation under physical stress evidently occurs as the result of the activation of glutaminase, which causes deamination of glutamine (Simakova, 1954).

Hyperoxia and Hypoxia. Oxygen intoxication is accompanied by an increase of the ammonia content in the brain. It was shown in experients with white rats that the effect of 4 atmospheres of oxygen leads to a nearly 15-fold increase of the ammonia content in the brain. Thus, if the ammonia level in the brain of intact animals is 0.27 (0.08-0.09) μM/g, it will rise to 4.03 μM/g in oxygen intoxication (Gersenovich, Krichevskaya and Bronovitskaya, 1957). Hypoxia, on the other hand, inhibits ammonia formation. It was shown in experiments on rabbits that hypoxia (an altitude of 7,500-8,500 m) leads to a drop in the ammonia content in the tissues of the organism. The ammonia content in the skeletal muscles of rabbits is 2.4 mg%; following the effects of hypoxia, it drops to 1.6 mg% (Simakova, 1954).

Effect of Pharmacological Substances. There is considerable data available on the effect of various pharmacological substances on the level of ammonia in the organism. Among the most important of these are such amino acids as glutamic and α-ketoglutaric acids. These acids are able to bind free ammonia in the organism to form glutamine and glutamic acid, respectively. Evidently α-ketoglutaric acid is more effective. The ammonia level in the blood of patients suffering from cirrhosis of the liver rises from 25-30 to 35-120 γ%; injection of 4-10 g of calcium α-ketoglutarate daily for 35 days allows a pronounced therapeutic effect to be acheived (Deuil, Godard and Gentilhomme, 1964). Derivatives of glutamic acid, N-acetyl glutaminate and N-carbamyl glutaminate, reduce the ammonia level in white mice and rats with hyperammonemia produced by intraperitoneal injection of ammonium chloride (Chiosa, Niculescu, Bonciocat and Stanaiv, 1965). A protective effect in /41
ammonia poisoning is also produced by dl-carbamyl aspartic acid (Cittadini, Cristofaro, Balestrieri and Cimino, 1966). It was shown in experiments with rats that injury to the liver by ethionine leads to an increase in the ammonia level in the blood to 180 γ%. Under these conditions, the introduction of nialamide-inhibitor monoaminoxidase reduces the ammonia content to 120 γ%. This same preparation reduces the ammonia level in dogs with hyperammonemia produced by the injection of a 2% solution of ammonia (Zuidema, Kirsh, Cares, Kowalczyk and Coon, 1963).

Data on the effect of radioprotectors, whose use is possible under the conditions of space flight, on the ammonia level in the brain are very interesting, It has been shown that aminothiols did not change (but indolealkyl amides reduced) the ammonia content in the brains of white rats (Strelkov, 1967).

Attempts have been made to achieve reduction of the ammonia level in the blood with the aid of injections of the urease inhibitor 'khlorlizodrin.' These attempts were unsuccessful (Zuidema, Sherman, Cullen and Kirsh, 1964). In fact, urease inhibitors are valuable when used to retard the breakdown of urea (with liberation of ammonia) under the influence of this enzyme in standing or stored urine, but not under *in vivo* conditions, when ammonia formation due to the decomposition of urea does not play a significant role.

Carbon Monoxide

Ionizing Radiation. The effect of ionizing radiation on the endogenic formation of carbon monoxide was studied in detail in a series of papers by V. V. Kustova et al.

These authors showed that irradiation of white rats by X-rays in a dose of 1,000 r produces an increase in the level of carboxyhemoglobin in the blood of these animals by 136% on the 7th day of radiation sickness. Irradiation of human blood *in vitro* leads to an increase in its carboxyhemoglobin content by nearly 2 times (Kustov, Troshanova, and Ivanova, 1960). The authors suggest that the cause of the increase of the carboxyhemoglobin content in the blood of irradiated animals consists in increased catabolism of hemoglobin under the influence of peroxide compounds formed in the process (Kustov, Gofman, and Ivanova, 1961). It is also evidently contributed to by the drop in the activity of blood catalase, which occurs following the action of X-rays (Tiunov, Vasil'yev, and Smirnova, 1962). A drop in the activity of catalase is accompanied by a rise in the formation of bile pigments. This was shown by Haeger-Aronsen in 1962 in experiments with rabbits in which the catalase activity was suppressed by allylisopropyl acetylcarbamide. If animals are injected prior to irradiation with a ferruginous complex of triethyl tetramine, which has a catalase-like effect (Wang, 1955), the drop /42
of activity of the catalase can be prevented (Smirnova, 1962). This same preparation, injected into rabbits prior to irradiation or added to blood from healthy subjects, irradiated *in vitro*, prevented increased formation of carboxyhemoglobin under the influence of ionizing radiation (Kustov, 1962). These data are indirect proof of the fact that an increase in the endogenic formation of carbon monoxide under the influence of ionizing radiation is the consequence of increased hemoglobin degradation. Some researchers, however, have not been able to detect endogenic formation of carbon monoxide under the influence of radiation (Vysochina, 1963).

In this regard, in order to clarify the problem of how X-radiation affects the intensity of carbon monoxide excretion from the organism to the surrounding atmosphere, we conducted special joint experiments with T. S. Kolosova using white rats.

Twenty male white rats weighing 180-200 g were placed in a hermetically sealed chamber. The temperature, humidity, and oxygen and carbon monoxide levels in the chamber were maintained at a constant level. The carbon monoxide level was measured every 24 hours for 96 hours.

These animals were then irradiated with X-rays in the amount of 700 r; 5 days later, they were again placed in the hermetically sealed chamber for 96 hours (Table 8). This experiment shows that the intensity of carbon monoxide excretion by irradiated animals is higher than in healthy non-irradiated animals.

TABLE 8. EFFECT OF X-RADIATION (700 r) ON THE INTENSITY OF CARBON MONOXIDE EXCRETION BY WHITE RATS.

Duration of stay in hermetically sealed chamber, hours	Carbon monoxide in air of hermetically sealed chamber, mg/m^3	
	intact	5 days after irradiation
24	25	25.0
48	28.0	40.0
96	30.0	39.0

Hypoxia. Hypoxemic states are accompanied by an increase in the endogenic formation of carbon monoxide. Excretion of carbon monoxide is increased by breathing air containing 9% oxygen for 10 minutes. Similar results are obtained after breathing a gas mixture containing 48% carbon dioxide for 30 minutes. The endogenic formation of carbon monoxide is thereby increased 5 times. The authors of these papers state that under /43
the influence of hypoxia there occurs in the organism an accumulation of incompletely oxidized products, a shift of the pH to the acid side and (as a consequence of the latter) increased degradation of hemoglobin and increased production of endogenic carbon monoxide (Malmstroem and Sjoestrand, 1953). However, not just any degree of hypoxia will increase the endogenic formation of CO. In the observations made by Biget (1954) of 7 men in a chamber where the oxygen content was reduced and corresponded to that at a height of 4,000 m, it was not possible to detect any increase in the endogenic formation of carbon monoxide.

Physical Stress. Heavy physical work causes increased endogenic formation of carbon monoxide (Malmstroem and Sjoestrand, 1953). Graziani and Mungo (1956), who studied 20 men after heavy physical work, detected carbon monoxide in the blood plasma.

Illness. The greatest number of reports in the literature deal with increased endogenic formation of carbon monoxide in human subjects suffering from various forms of anemia (Leoper, 1939; Sjoestrand, 1950; Bakowa and Szezepkowski, 1960). An increase was observed in the endogenic formation of carbon monoxide in patients with cardiovascular diseases, diabetes, and infections of the respiratory organs (Belli and Giuliani, 1955; Giobelo and Parmeggiani, 1959). Increased formation of carbon monoxide has been described in patients ill with silicosis (Cavigneaux, Fuchs and Tara, 1949). Data are also available on the intense endogenic formation of carbon monoxide in cases of burns (Troell, Norlander and Johanson, 1955). Similar data on the endogenic formation of carbon monoxide in various kinds of diseases are found in the survey by Engstedt (1957).

CHAPTER IV

CHARACTERISTICS OF GASEOUS PRODUCTS OF HUMAN VITAL ACTIVITY

The chemical composition of the gaseous products of "human origin," /44
called anthropotoxins (Anthropotoxin mild) by Dubois Ramon, is very complex.

At the present time, with the aid of modern methods of investigation (gas chromatography, infrared spectrometry, mass spectrometry), more than 400 compounds have been found in the contents of the "metabolic excreta" of man; they represent 22 chemical groups of organic and inorganic substances (Weber, 1963).

Some of these compounds are formed in the organism in the process of normal metabolism and enter the surrounding medium with the expired air, urine or feces or are included in the composition of the excreta of sudori-sweat and sebaceous glands. A certain percentage of the gaseous chemical compounds of "human origin" are released into the atmosphere as the result of complex physical and chemical conversions and bacterial decomposition of various organic compounds contained in the urine, feces, sweat, cutaneous fat, etc. The chemical nature of both the endogenic and secondary gaseous products of vital quantities of gaseous excreta of "human origin" complicates estimation of their value in the formation of atmospheres in hermetically sealed environments. This fact has necessitated special studies to determine the amounts of the basic gaseous impurities excreted by man into the ambient medium and to determine the nature of their biological effect on the organism.

Characteristics of Certain Gaseous Toxic Substances Given Off By Fresh Urine

A practically healthy individual excretes 1,000-1,600 ml of urine daily, containing approximately 95% water and 5% organic and inorganic substances. A list of these products and the amount of each in grams in the daily urine are given below:

Chemical Composition	After Sinyak & Chizhov (1964)	After Mangelsdorf (1959) /45
Water	Approx. 1,400	1,200
Solid matter	--	60
Organic substances	22-46	--
Urea	20-35	30
Uric acid	0.2-1.2	0.7
Hippuric acid	0.1-2.5	0.7
Creatinine	0.6-1.9	1.2
Indican	--	0.01
Allantoin	--	0.04
Nitrogen from amino acids	--	0.2
Purinic bases	--	0.01
Phenols	0.0173-0.42	0.2
Acetone	0.009	--
Acetic acid	0.041-0.06	--
Formic acid	0.014-0.036	--
Citric acid	0.2-1.0	--
Oxalic acid	0.015-0.03	0.02
Inorganic substances	12-25	--
Chlorides (in the form of NaCl)	8-12	12.0
Na	--	4.0
K	--	2.0
Ca	--	0.2
Mg	--	0.15
Sulfur in the form of SO_2	--	2.5
Inorganic sulfates (SO_3)	--	2.0
Bound sulfates (SO_3)	--	0.2
Neutral sulfur	--	0.3

These data on urine composition are far from complete, however. The most detailed information, contained in the Biology Data Book (F. Altman, Ditmer, 1964) speaks of the presence in human urine of 229 chemical compounds. These include 103 nitrogenous compounds, 30 electrolytes, 22 vitamins, 38 hormones and 10 enzymes, as well as organic acids of lipids of hydrocarbons. In other sources, 183 chemical substances are listed in the composition of urine (Weber, 1967). The most important role in the formation of the gaseous com-

[4] [Tr. Note: Footnote not referred to in text]. The amount of organic substances in various daily portions of urine amounts to 13,000-36,000 mg O_2/liter. The amount of urea varies from 7 to 18 g/liter (Kozvrevskaya et al., 1967).

ponent of hermetically sealed systems is played by those component parts of urine which possess sufficient volatility. These include primarily ammonia, amines, and organic acids (Clemedson, 1959). The amines in urine include trimethylamine (Starck et al., 1963), dimethylamine (Dargel, 1966), ethylamine (Sakato, 1950; Perry, Show, Walker and Redlich, 1962), methylamine, ethylamine, propylamine, butylamine, ethanolamine (Davies, Wolf and Perry, /46
1954). Chromatographic studies have made it possible to isolate 40 different amines from urine, 26 of which have been identified. These include, in particular, methylamine, dimethylamine, ethylamine, ethanolamine, β-oxypropylamine, piperidine, putrescine, cadaverine, histamine, N-acetylhistamine, methylhistamine, p-oxybenzylamine, tyramine, 5-methoxytryptamine, normethanephrine, methanephrine, tryptamine, bufotenine and kynuramine (Perry and Schroeder, 1963).

In addition, this group of urine components must include acetone, phenols, paracresol (Lebber et al., 1966) methane, hydrogen, hydrogen sulfide (Slager, 1962), acetaldehyde, isoprene, ethanolmethyl formate, propionic aldehyde, methyl ethyl ketone, diacetyl, dimethyl sulfate, acetonitrile (McKee, 1960). Quantitative data on the urine content of certain chemical substances which are potentially able to enter the surrounding atmosphere are given in Table 9.

The materials on the content of many volatile products in the urine are very contradictory. They provide no possibility whatever of calculating the amount of any gaseous substance which is capable of being excreted into the surrounding atmosphere. This fact is the only thing that complicates the evaluation of the hygienic significance of the gaseous substances given off by stored urine. We therefore performed special studies in which we determined the amounts of several toxic substances given off by urine (Kustov et al., 1967).

In these tests, the content of toxic material was determined in a current of previously purified air, flowing for 3 hours through a glass container with 200 ml of freshly collected urine.

TABLE 9. CONTENT OF CERTAIN HIGHLY VOLATILE CHEMICAL SUBSTANCES IN HUMAN URINE.

Substance	Content	Source
Ammonia	40-80 mg/%	Maksimova, 1951
Ammonia	0.8317 g**, ages 16-18	Podrabinik, 1953
Ammonia	0.3822 g**, ages 25-35	
Ammonia	0.5624 g**, ages 50-62	
Ammonia	0.574-0.868 g**	Mazurina, 1939
Acetone	0.155%	Mason, Enstrom, 1950
Dimethylamine	0.5-4.0 mg**	Levin, 1955
Ketonic bodies	0.9-2.8 M/l	Johnson, Sargent, Passmore, 1958
Ketonic bodies		
p-Cresol	0.285 mg**/kg wt.	Spector, 1956
p-Cresol	45.7 mg/l	Bergerova-Fiserova, 1954
p-Cresol	87 mg(65-117)**	Schmidt, 1942
p-Cresol	1.8 mg %	Siegfried, Zimmerman, 1911
Methyl alcohol	0.3 mg %	Leaf, Zatman, 1952
Ethyl alcohol	>0.2 mg %	Buecher, Redetzki, 1951
Ethyl alcohol	>0.1 mg %	Walker, Curry, 1966

* The daily urine of dogs contains 0.005-0.95 g of ammonia (Zilov, 1948).
** Daily Urine.

The results of the experiments are listed in Table 10.

It follows from the data in the Table that air that has passed through fresh urine always contains ammonia with aliphatic amines and phenols. Frequently, one also finds ketones, organic acids, and very rarely, hydrogen sulfide with mercaptans. The gaseous excreta contain large amounts of organic compounds ("hydrocarbons"). It is striking that carbon monoxide is excreted from fresh urine[5].

[5] The possibility of separating carbon monoxide from urine was demonstrated in the experiments of Pecora et al., (1959).

TABLE 10. LIBERATION INTO THE ATMOSPHERE OF TOXIC SUBSTANCES FROM FRESH URINE

Amount in mg/100 ml urine					Amount in mg/liter air	
Ammonia and aliphatic amines	Phenols	Mercaptans and H_2S	Ketones converted to acetone	Organic acids converted to CH_3COOH	Carbon monoxide	Carbohydrates converted to methane
0,069	0,014	--	—	—	0,068	0,017
0,023	0,012	—	--	—	0,140	0,038
—	0,029	Traces	—	—	0,015	0,055
0,014	0,041	—	0,032	—	0,076	0,083
0,002	0,026	—	0,009	—	0,046	0,031
0,007	0,045	--	0,029	—	0,070	0,005
0,004	0,033	—	0,005	—	0,091	0,010
Traces	0,013	--	Traces	0,033	0,020	0,021
»	0,017	--	0,001	--	0,006	--
»	0,013	--	Traces	Traces	0,003	—
»	0,016	—	»	»	—	—
M 0,012	0,024	—	0,010	—	0,055	0,033

Commas indicate decimal points.

Simple calculations will show that when the atmosphere of a standard cabin[6] contains ammonia, acetone, phenols, CO, and carbohydrates in the average amounts listed in Table 11, the concentration of each substance will be several factors less than its permissible level for the atmospheric medium of working quarters (Table 11). It was possible to assume, however, that these concentrations are hygienically significant under the influence of an entire complex of gaseous substances given off by fresh urine. /48

The above assumption was tested by us and by V. I. Mikhaylov and L. T. Poddubnaya in special experiments. The conduct of studies of this kind was all the more justified by the fact that the values of the permissible concentrations listed in Table 11 apply to the individual substances and not to the complex of chemical compounds given off by fresh urine.

[6] A standard cabin is understood to be a hermetically sealed chamber in which there is one person and which has a free volume of air of 1 m^3 (Clemedson, 1959).

TABLE 11. POSSIBLE CONTENT OF SEVERAL CHEMICAL SUBSTANCES GIVEN OFF BY FRESH URINE IN THE ATMOSPHERE OF A STANDARD CABIN.

Substance	Possible concentration (after 3 hrs, mg/m^3)	Maximum permissible concentration, government standard 245-63, mg/m^3
Ammonia	~0.012	20
Phenols	~0.024	5
Acetone	~0.01	200
Carbon monoxide	~0.055	20
Hydrocarbons converted to C	~0.033	300

To study the nature of the biological effect of the complex of gaseous toxic substances given off by fresh urine, we conducted experiments on male white mice weighing 20-25 grams. The animals were placed in glass desiccators with a volume of 8 liters. Room air, previously purified in a system of columns with pumice, impregnated with sulfuric acid, alkali, silica gel and hopcalite, was bubbled through fresh urine (200 ml) at the rate of 1 liter/min and admitted to the animals' chamber. The time of exposure to the complex of chemical substances listed below (in mg/liter) was 2 hours.

Ammonia and aliphatic amines	0.0022
Acetone	0.0005
Aldehydes	Traces
Organic acids converted to acetic	0.0044
Nitrogen oxides converted to N_2O_5	Traces
Carbon monoxide	0.029
Hydrocarbons converted to carbon	0.038
Phenols	0.0006
Oxygen (in %)	20.7
Carbonic acid (in %)	0.4

/49

During the exposure time, the behavior of the animals was observed; after it was over, the activity of cholinesterase in the blood was determined by the method of A. A. Pokrovskiy (1953) and that of catalase by the method of A. N. Bach and S. R. Zubkova (1950); the content of acetylcholine in the blood was determined by the method of Khestrin as modified by L. Ya. Livshits and B. Rubin (1961), and the level of carboxyhemoglobin (HbCO) was determined by the Wolf method (1959).

These experiments showed that the complex of toxic substances given off by fresh urine produced a reaction in the experimental animals that was characteristic of the effect of irritant gases. The white mice were observed to be animated and restless; they lost their appetites and closed their eyes; the animals' respiration rate was seen to increase.

Examinations of the experimental animals failed to reveal any significant changes in the HbCO content of the blood or in catalase activity (Figure 4).

In addition, we observed a noticeable increase of the acetylcholine content in the blood and increased activity of cholinesterase (Figure 5), which clearly indicates some stress on the physiological system that produces a state of neurohumoral compensation in the organism (Al'pern, 1963).

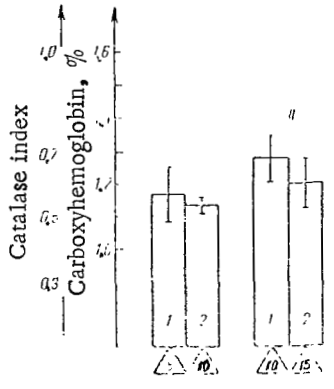

Figure 4. Effect of Toxic Substances Given Off by Fresh Urine on Catalytic Activity (I) and the Content of Carboxyhemoglobin in the Blood of White Mice (II). 1, Control; 2, Experimental. In triangles: number of animals.

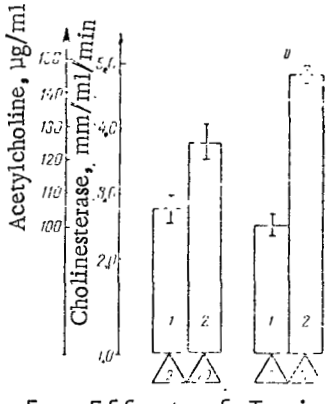

Figure 5. Effect of Toxic Substances Given Off by Fresh Urine on the Amount of Acetylcholine (I) and Activity of Cholinesterase (II) in White Mice. Symbols same as in Figure 4.

All of this supports our view (mentioned earlier) that the gaseous chemical substances given off by fresh urine in relatively small amounts, in the event of their joint action on an organism, can produce not only a stimulatory but even a generally toxic effect.

Characteristics of Certain Gaseous Toxic Substances Given Off by Stored Urine

Human urine is a favorable nutrient medium for various microorganisms. During their vital activity, there is decomposition of the organic and inorganic compounds in the urine with liberation of various chemical substances into the surrounding air. The majority of these substances do not differ from the chemical products given off by fresh urine. However, some of these substances may belong to other chemical groups. /50

All of these gaseous products may become dangerous in the event of disturbance of the hermetic seals of the special containers for storing urine aboard spacecraft.

To estimate the degree of this danger, we determined the content of toxic substances in the gaseous products of stored urine (Kustov, Mikhailov, Poddubnaya, 1967) and studied their biological effects.

In the experiments, we attempted to trace the change in the composition and amount of toxic substances given off by urine during storage for 3.5 and 10 days at a temperature of 18-20°C. Collected urine in the amount of 500 cm^3 was stored in 5-liter hermetically sealed glass bottles. At the end of the specified storage period, samples of air were drawn from the bottles for subsequent chemical analysis (Table 12).

It is clear from the Table that the gaseous products of stored urine include ammonia and aliphatic amines, ketones, organic acids, carbon monoxide, hydrocarbons and phenols, i.e., the same substances that we observed in the gaseous products of fresh urine. In addition to the substances listed, the air above the stored urine was found to contain some gaseous products which were not found among the gaseous products of fresh urine. This includes (in particular) nitrogen oxides, hydrogen sulfide with mercaptans, and sulfide gas.

The data shown in Table 12 also indicate that as the storage period of the urine increases, the liberation into the air of ammonia with aliphatic compounds likewise increases, as do the amounts of organic acids, hydrocarbons, and nitrogen oxides. /51

TABLE 12. GASEOUS CHEMICAL SUBSTANCES (in mg/100 ml) GIVEN OFF BY URINE DURING STORAGE.

Substance	3-day urine			5-day urine			10-day urine		
	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
Ammonia and aliphatic amines	0,094	Traces	0,014/7	0,047	0,001	0,019/10	0,0125	0,005	0,061/7
Ketones	0,002	Traces	0,0006/6	0,014	0	0,008/12	0,007	Traces	0,001/7
Organic acids converted to acetic acid	0,058	0,012	0,028/6	0,111	0,047	0,076/5	0,49	0,018	0,139/6
Hydrocarbons converted to C	0,151	Traces	0,094/6	0,38	0,005	0,079/8	0,328	0,031	0,173/4
Phenols	0,008	0,005	0,006/7	0,012	0,004	0,007/3	0,0035	0,0005	0,0019/0
Nitrogen oxides converted to N_2O_5	0,003	Traces	0,0013/5	0,0021	0	0,001/6	0,036	Traces	0,015/6
Nitrogen sulfide and mercaptans	—	—	—	Traces	0	—	Traces	—	—
Sulfur dioxide	—	—	—	—	—	—	Traces	—	—
Carbon monoxide	0,071	0,017	0,035/6	0,016	0,00[illegible]	0,012/5	0,056	Traces	0,018/7

Note: Numerator = substance, in mg; denominator = number of determinations from which the average was drawn.

Commas indicate decimal points.

In a bacteriological study of the stored urine, intensive multiplication of various urobacteria was observed in it. By the 10th day of the experiment, the number of microbial bodies in 1 ml of urine increased from 90.5 ± 15 to $4.8 \cdot 10^9 \pm 1.10^{-9}$. All of this allows us to attribute the increased production of these gaseous substances and the(appearance in the air above stored urine of nitrogen oxides, sulfur anhydride, and sometimes hydrogen sulfide, due to microbial decomposition of the organic and inorganic products contained in urine.

The differences in the characteristics of the gaseous products of fresh and stored urine were not reflected in the nature of their biological effects. In our further studies, then, conducted jointly with V. I. Mikhaylov and L. T. Poddubnaya, we studied the overall toxic effect of the complex of toxic substances given off by 10-day old urine. /52

The method of conducting these experiments did not differ from the arrangement of the tests for studying the features of the combined effect of gaseous products from fresh urine. The content of chemical substances (in mg/liter) in the air of the experimental chamber, after bubbling it through 10-day old urine, reads as follows:

Ammonia and aliphatic amines	0.0327
Acetone	0.0007
Aldehydes	Traces
Organic acids converted to acetic acid	0.085
Mercaptans and hydrogen sulfide	Traces
Nitrogen oxides converted to N_2O_5	0.007
Carbon monoxide	0.024
Sulfide gas	Traces
Hydrocarbons converted to C	0.093
Phenols	0.0014
Oxygen (in %)	20.7
CO_2 (in %)	0.87

Admission of this mixture of gases into the experimental chamber caused the animals to become agitated and increase their rate of respiration. Signs of irritation of the eyes and upper respiratory pathways were observed.

Following a 2-hour exposure, the animals did not show any change in the level of HbCO in the blood or in their catalase activity. In addition, we observed an increase in the content of acetylcholine in the blood with practically constant activity of cholinesterase (Figure 6). This kind of shift is one of the symptoms of insufficiency of the physiological system ensuring neurohumoral equilibrium in the organism (Al'pern, 1963).

Since the gaseous products of fresh urine merely placed a stress on this system, the signs of its insufficiency that were observed in experiments with stored urine obviously may be an indicator of a more pronounced toxic effect of the gaseous products produced in the experiments. The observed effect may be caused by a large (relative to the gaseous products of fresh urine) content in the air of the chamber of ammonia and aliphatic amines, organic acids, hydrocarbons and phenols, as well as the presence in the mixture of small amounts of nitrogen oxides, hydrogen sulfide and sulfide gas, which were completely absent from the air above the fresh urine. It should

be mentioned that the biological action of the complex of substances given off by 10-day old urine was obtained only after a 2-hour exposure, while the effect of the volatile products of fresh urine lasted 3 hours. /53

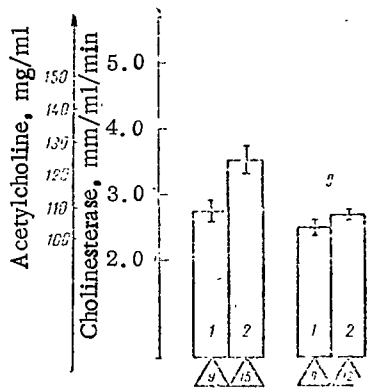

Figure 6. Effect of Toxic Substances Given Off by Stored Urine on the Content of Acetylcholine (I) and Activity of Cholinesterase in the Blood (II) of White Mice. Symbols same as in Figure 4.

All of the above leads us to conclude that the complex of gaseous products from stored urine may harbor greater potential danger than gaseous products from fresh urine.

Thus, urine that is stored in containers of small volume in special areas is a potential source of air pollution by a number of toxic substances that have an unpleasant odor and an irritating and generally toxic effect.

The presence of even small amounts of these substances in the atmosphere of a space with a small volume can have a negative effect on the working ability and general state of health of an individual. Thus, for example, during the flight of the American spacecraft "Gemini 3", Astronaut McDivitt reported to Mission Control that it was extremely unpleasant for him to breathe in the odor of decomposing urine.

In view of the fact that the majority of the gaseous toxic substances are formed by the microbial decomposition of organic products contained in urine, one way to reduce the intensity of gas production would be to find a method of treating the excreted urine with various antiseptics.

For this purpose we can use chemical compounds from the phenol and alcohol groups as well as the salts of heavy metals, etc. The relative effectiveness of some of these antiseptics (germicides) was studied by Putnam (1965).

The ions of the heavy metals (Cu, Fe, Ag, Co, Hg, etc.) preserve the sterility of urine for 2-9 months (depending on the concentration of the metal) and the oxidizing agents (CrO_3, H_2O_2, HOCl, NaOCl, $LiNO_3$, $KMnO_4$, $HClO_4$, $Ca(ClO)_2$, $4H_2O$, etc) do the same for periods of 2 days to 2 weeks.

Of the many antiseptics of equivalent effectiveness, Putnam preferred chromium oxide (CrO_3); whose bactericidal properties were roughly equal to /54
those of the ions of the heavy metals and oxidizing agents. In addition, chromium oxide in the presence of H_2SO_4 did not produce a precipitate that could block the tubing of a urine regeneration system. Other compounds tested by the author were either less effective antiseptics than CrO_3 or else their presence provoked the precipitation of uric acid salts.

In 1965-1967, L. N. Rogatina conducted detailed studies to find effective chemical preservatives for urine. In experiments *in vitro*, she determined the antiseptic properties of several salts of heavy metals, phenol-containing compounds, antibiotics, inorganic and organic acids, quaternary-ammonium bases and a number of other chemical substances. A total of 56 preparations were tested. The most effective urine preservatives (judging by their minimal effective dose) were: a preparation from a group of phenols, copper sulfate, copper chloride, thiuram, 'katapine' "K" and brilliant green; less activity was noted in phenyl trichloracetate, boron trichloride and preparations of silver and hexachloraphene.

One of the preservatives selected by L. N. Rogatina was used in our laboratory in studying the problem of the effect of chemical antiseptics on the intensity of the production and liberation into the air of the toxic substances from stored urine. The experiments were conducted by L. N. Rogatina, L. T. Poddubnaya and V. I. Mikhaylov.

Collected urine (2 l) was stored in two 20-liter hermetically sealed glass bottles. Preservative was added to one of the bottles in the amount of 2.5 g of preparation to 1 liter of urine.

Prior to addition of preservatives and after the end of the storage period (10 days), the urine was examined for the presence of microorganisms,

as well as their number and type. On the tenth day of the experiment, samples of air were drawn from the bottles for subsequent chemical analysis.

The experiments that were performed indicated that on the tenth day of storage the number of microorganisms in urine to which no preservative had been added had increased from tens to billions of microbial bodies per ml. Addition of preservative to the urine almost completely halted growth of the organisms. The preservation effect in this case was practically 100% (Table 13). /55

TABLE 13. NUMBER OF MICROORGANISMS IN 1 ml OF URINE STORED FOR 10 DAYS (AVERAGE DATA FROM 8 EXPERIMENTS).

Data	Without preservative	With preservative
Original level	90.5 ± 15	774.2 ± 85.3
After 10 days	$4.8 \cdot 10^9 \pm 10^9$	6.25 ± 3.0

Data on the effect of the preservative on the intensity of the production and liberation of several toxic substances from 10-day urine are shown in Table 14.

It is clear from the Table that the addition of a preservative reduced the liberation of ammonia and aliphatic amines, acetone, volatile organic acids, nitrogen oxides and phenols into the air above the stored urine, and did not have any noticeable effect on the liberation of carbon monoxide and organic compounds, determined *in toto* by carbon.

Calculations show that in comparison with background tests the preservative in question reduced the liberation into the air of substances from the ammonia group by a factor of 47 on the average, from the acetone group by about 1.4 and from the fatty acids by more than 9. As a rule, nitrogen oxides were present in the air above the urine in trace amounts, while in the control experiments their content in 5 samples out of 8 varied from 0.004 to 0.036 mg/liters.

TABLE 14. EFFECT OF PRESERVATIVE ON THE INTENSITY OF EMISSION OF CERTAIN GASEOUS TOXIC SUBSTANCES BY URINE STORED FOR TEN DAYS.

Amount of chemical substances, mg/100 ml urine													
Without preservative							With preservative						
Ammonia and aliphatic amines	Ketones	Organic acids converted to acetic acid	Nitrogen oxides converted to N_2O_5	Carbon monoxide	Hydrocarbons converted to carbon	Phenols	Ammonia and aliphatic amines	Ketones	Organic acids converted to acetic acid	Nitrogen oxides converted to N_2O_5	Carbon monoxide	Hydrocarbons converted to carbon	Phenols
—	0,001	0,129	—	—	—	—	0,0025	0,0008	—	0,0034	0,028	—	0,0015
0,053	0,007	0,083	0,018	0,012	0,018	0,0010	0,0047	0,0010	0,009	0,0025	0,028	—	0,0011
0,016	0,0015	0,490	0,012	0,029	—	0,0035	0,0011	0,0006	0,007	Traces	0,034	0,057	0,0006
0,056	0	0,035	0,006	0	—	0,0030	0	0,0013	0,016	»	0,014	0,014	0,0007
0,005	0,0026	0,018	—	0	—	0,0012	0,0016	0,0004	0,031	0	—	0,164	0,0007
0,125	0,0009	—	0,036	0,012	0,151	0,0005	0	0,0005	0,014	Traces	—	0,204	0,0007
0,077	0,0013	0,080	0,004	0,028	0,031	0,0026	0	0,0005	0,007	»	0,024	0,300	0,0007
0,093	0	—	Traces	0,050	0,0328	—	0	0,0005	0,016	»	0,014	0,170	0,0006
M 0,061	0,001	0,139	0,015	0,018	0,173	0,0019	0,0013	0,0007	0,014	—	0,016	0,148	0,0008

Commas indicate decimal points.

If we compare these data with the bacteriostatic effect of the preservative, we can conclude that production of the gaseous chemical substances listed above depends mainly on microbial decomposition of urea and other organic compounds which go to make up urine. As far as the carbon monoxide is concerned, its liberation from stored urine is clearly not related to the vital activity of the microorganisms, since introduction of a preservative had no significant effect on its production.

Hence, these experiments showed that with the aid of a preservative it is possible to achieve significant reduction of the liberation of several toxic substances from stored urine and simultaneously to reduce potential danger from the gases liberated from urine as a whole.

Characteristics of Gaseous Chemical Substances Given Off By Feces

The amount of feces excreted by an individual depends on the nature and amount of ingested food, the functioning of the gastrointestinal tract and the peculiarities of metabolism, and amounts to 250-2,300 g/day. Fecal matter contains about 75% water; 25-30% of its weight comes from bacteria that are parasitic in the intestine, while the remainder consists of undigested food (16-20% of the dry substance of the feces) and other materials. The feces contain a considerable amount of products of protein metabolism--amino acids, etc., lipids, neutral fats, and free fatty acids (about 2 g/day); bile pigments and the products of their decomposition, stercobiligen and fecal stercobilin (about 0.5 g/day), some mineral substances--calcium, phosphorus, magnesium, iron, phosphates, aluminum, copper, mercury, nickel, sulfur, zinc, sodium (Altman, Dittmer, 1964). Human feces contain 0.02-0.16% of ammonia /57 (Goiffon, 1925). According to later data, the amount of ammonium nitrate in the feces is 0.36-120 mg per kg of body weight (Altman, Dittmer, 1964).[7]

[7] The content of total nitrogen in the daily portion of feces (average dry weight = 50 g) varies from 1,050 to 1,950 mg, the amount of ammonium nitrate from 2.5 to 500 mg, and the amount of nitrogen in nitrate form, from 0 to 160 mg (Kozyrevskaya, et al., 1967).

As a result of the vital activity of aerobic and anaerobic microorganisms in the feces, products appear which are usually not formed in the course of metabolism characteristic of man. For example, the microbial breakdown of cysteine contained in the feces leads to the formation of hydrogen sulfide; the breakdown of tryptophane liberates indole and skatole into the atmosphere, causing the characteristic odor of feces.

Under the influence of microflora in the intestine, there is breakdown and decarboxylization of amino acids to form various amines. Chromatographic analysis of fresh feces has determined the presence of the following: methylamine, dimethylamine, ethylamine, diethylamine, propylamine, butylamine, amylamine, phenylethylamine, tyramine, putrescine, cadaverine, ethanolamine, taurine and kynurenine. In addition, certain amino acids are formed: lysine, alanine and arginine (van Rheenen, 1962).

Microbial decomposition of tyrosine is observed to lead to the formtion of tyramine and phenol. In addition to the products listed above, the feces contain carbohydrates and the products of their breakdown by enzymes, the organic acids (acetic, lactic, and butyric). The feces always contain carbon dioxide gas, hydrogen, methane, paracresol, parahydoxyphenylpropionic acid and other chemical substances (Mangelsdorf, 1959). They also contain vitamins and compounds closely resembling them: thiamine, carotene, xanthophyll, biotin, riboflavin, ascorbic acid and Vitamin E (Altman, Dittmer, 1964). At the present time, modern methods of chemical analysis list a total of 196 chemical compounds in feces (Weber, 1967).

However, not all of the substances entering into the composition of feces are volatile and can enter the surrounding air. The list of chemical compounds that exhibit possible danger includes mainly such gaseous substances as amines, organic acids, phenols, hydrogen sulfide and mercaptans, indole, skatole and some other substances.

Data on the amounts of these substances in the air above feces are limited. According to Clemedson (1959), 100 g of feces contain about 60 mg of indole, and the amount of it generated by the daily output of feces is about

90 mg. Therefore, we carried out special studies in our laboratory to determine the amounts of the basic gaseous substances that can enter the atmosphere from freshly excreted human feces (Kustov et al., 1967). /58

The methodology of these experiments was the following: freshly collected human feces were placed in hermetically sealed glass desiccators with a volume of 8 l. In the air of one of the desiccators, we determined the amount of ammonia and its compounds, hydrogen sulfide and mercaptans, phenols, volatile organic acids, indole and skatole. To do this, all of the air from the desiccator was evacuated by a blower through a system of chemical absorbers. As the air in the desiccator became more rarefied, room air was admitted after first being cleaned in columns with solid alkali, silica gel, pumice, saturated sulfuric acid and hopcalite. The intake tubes in the desiccator were arranged so that the purified air blew over the feces. Air samples were collected 2 hours later.

A second desiccator was kept closed for 2 hours (to obtain comparative results). Then the air from it was collected in evacuated containers for subsequent determination of the amounts of carbon monoxide, hydrocarbons, nitrogen oxides, oxygen and carbonic acid.

The determination of the amounts of harmful substances was done by standard methods used in industrial and sanitation chemistry (Alekseyeva et al., 1954; Peregud and Gernet, 1965;). The content of toxic substances was calculated on the basis of 100 g of freshly excreted feces (Table 15). /59

It is clear from the Table that the content of toxic substances listed in each of the 11 experiments was subject to significant variations, apparently related to the content of bacterial flora in the intestines of the various subjects and the manner of collection of the feces.

In addition, the data obtained show that during the first two hours freshly collected feces give off considerable amounts of hydrocarbons, carbon monoxide, volatile organic acids, nitrogen oxides, phenols, and also ammonia and aliphatic amines. Mercaptans and hydrogen sulfide were found in trace amounts. Indole and skatole were found in 5 cases out of 11.

TABLE 15. AMOUNTS OF CERTAIN TOXIC SUBSTANCES GIVEN OFF INTO THE AIR BY FRESH FECES (in mg/100 g).

Ammonia and aliphatics amines	Mercaptans and hydrogen sulfide	Phenols	Indole and skatole	Organic acids converted to acetic acid	Nitrogen oxides converted to N_2O_5	Hydrocarbons converted to CH_4	Carbon monoxide
0,052	Traces	0,011	0	Traces	0,007	0,120	—
0,035	»	0,009	0	—	0,005	0,248	0,023
—	0	0,007	0	0,079	0,042	1,780	0,081
—	0	0,006	0	0,138	0,003	0,696	0
0,008	Traces	0,013	0	0,111	0,003	0,173	0
0,062	»	0,010	Traces	0,155	Traces	0,696	0
0	»	—	0	0	0,078	—	0,170
0	0	—	Traces	0,203	0,094	—	0,494
0,011	0	0,011	0,007	0,310	0,236	1,230	0,330
0	Traces	0,006	0,008	0,846	0,123	1,430	0
0	0,113	—	0,013	0,500	0,007	—	—
M 0,019	—	0,009	—	0,259	0,061	0,802	0,122

Commas indicate decimal points.

If the amounts of toxic substances listed in the Table were allowed to enter a standard cabin for 2 hours, their possible content in the atmosphere could reach the values shown in Table 16.

TABLE 16. POSSIBLE CONTENT OF CERTAIN CHEMICAL SUBSTANCES GIVEN OFF BY FRESHLY COLLECTED FECES IN THE ATMOSPHERE OF A STANDARD CABIN.

Substance	Concentration which can be reached in 2 hrs., mg/m³	Limit of attainable concentration of CH_{245}, mg/m³	Substance	Concentration which can be reached in 2 hrs., mg/m³	Limit of attainable concentration of CH_{245}, mg/m³
Ammonia and aliphatic amines	~0,019	20	Organic acids converted to acetic acid	~0,259	5
Mercaptans and hydrogen sulfide	To 0,113	3	Nitrogen oxides converted to N_2O_5	~0,061	5
Phenols	~0,009	5	Carbon monoxide	~0,122	20
Indole and skatole	To 0,013	—	Hydrocarbons converted to carbon	~0,802	300

Commas indicate decimal points.

It follows from the Table that the possible content of each of the toxic substances in the atmosphere of the cabin that is included in the composition of the gaseous substances given off by 100 g of freshly excreted feces does not reach the limit of its attainable concentration. It was possible to suggest, however, that by their combined action on the organism, these concentrations (safe at first glance) could have a significant effect on the overall biological effect of the gases given off by freshly collected feces. We devoted our later experiments to testing this hypothesis.[8]

The experiments were performed on white male mice weighing 20-22 g. /60
The animals were placed in glass desiccators, to which purified room air that had been passed through a chamber containing freshly collected feces had been added in advance. The exposure of the animals to the gaseous products of the feces lasted 2 hours. The content of chemical substances (in mg/L) in these gaseous products was as follows:

Ammonia and aliphatic amines	0.0002
Aldehydes	Traces
Acetone	0.0002
Organic acids converted to acetic acid	0.02
Indole	0.0001
Mercaptans and hydrogen sulfide	0.0005
Nitrogen oxides converted to N_2O_5	Traces
Carbon monoxide	0.029
Sulfide gas	0.0004
Hydrocarbons converted to "C"	0.102
Phenols	0.0006
Carbon dioxide (in %)	Approx. 1
Oxygen (in %)	Approx. 20.2

To estimate the biological effect of this complex of substances on the animals, we determined the latent time of the flexor reflex and the same biochemical indices as in the experiments studying the features of the toxic effect of the gases given off by urine.

[8] The experiments were conducted by us in conjunction with V. I. Mikhaylov and L. T. Poddubnaya (unpublished data).

The experiments that were conducted showed that during the first 20-25 /61
minutes of exposure to all of the gaseous products from fresh feces the animals evidenced motor stimulation and an increased rate of respiration. The white mice lost their appetites and closed their eyes. Then the irritating effect of the mixture gradually decreased. About 1 hour after the beginning of exposure, the animals calmed down, sat immobile, and huddled in the corners of the chamber.

At the end of the exposure period, the animals showed a statistically reliable increase in the level of carboxyhemoglobin (Figure 8) and acetylcholine (Figure 7) in the blood. The activity of cholinesterase and catalase in the blood remained practically constant (Figure 9).

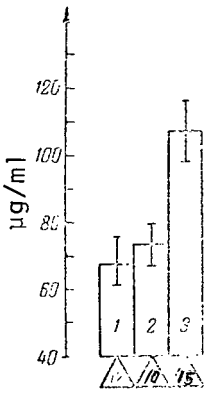

Figure 7. Effect of Toxic Substances Given Off by Freshly Excreted Feces on the Level of Acetylcholine in the Blood of White Mice. 1, Intact animals; 2, Before the effect; 3, After the effect. In the triangles: number of animals.

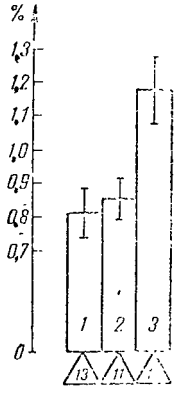

Figure 8. Effect of Toxic Substances Given Off by Freshly Excreted Feces on the Carboxyhemoglobin Content of the Blood of White Mice. Symbols same as in Figure 7.

The changes that were noted in the cholinesterase-acetylcholine system in the direction of some accumulation of the latter, as well as the increase in the latent period of the flexor reflex (Figure 10) that was observed in the experiments apparently indicates some subcompensation in the physiological system which ensures neurohumoral equilibrium in the organism.

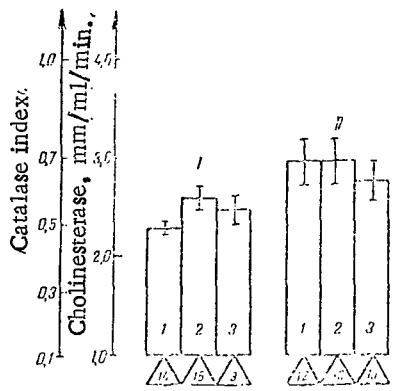

Figure 9. Effect of Toxic Substances Given Off by Freshly Excreted Feces on Catalase (I) and Cholinesterase (II) Activity in the Blood of White Mice. Symbols same as Figure 7.

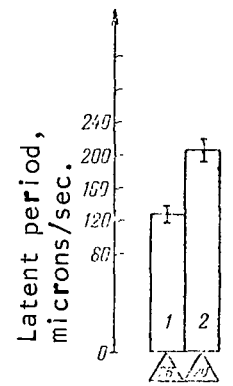

Figure 10. Effect of Toxic Substances Given Off by Freshly Excreted Feces on the Length of the Latent Period of the Flexor Reflex in White Mice. Symbols same as Figure 4.

All of this leads us to conclude that the toxic substances entering into the composition of the gaseous products given off by fresh feces, even though they occur in very small amounts, in their combined effect in the total mixture are not indifferent in their effect on the organism.

Hygienic Characteristics of Gaseous Chemical Substances Given Off by Stored Feces

The mineralization of the organic compounds that enter into the composition of excreted feces occurs in the course of complex biological, chemical and physical reactions. An important role in these transformations is played by the microorganisms which vegetate on feces. /62

The microbial decomposition of organic compounds of feces, beginning already in the intestines, is accompanied by the liberation into the surrounding medium of substances which are diverse in their chemical affiliations. Some of these substances belong to the very same chemical groups that we determined in previous experiments.

However, their content in the gaseous products from stored feces can increase significantly. In addition, it is possible for these gaseous

components to include some chemical substances which were not found in the air above fresh feces.

All of this can increase the potential danger of the gaseous products of feces, stored in special containers aboard modern aircraft.

In order to determine the degree of this danger, we determined the amount of certain gaseous chemical substances that enter the air from stored feces, and studied the biological effect of an entire complex of substances that enter into the composition of these gaseous products.

We conducted experiments in conjunction with V. I. Mikhaylov and L. T. Poddubna (cf. Kustov et al., 1967). The methodology of the experiments was the same as in the experiments in which we determined the amounts of certain gaseous substances in the air above fresh feces. The results of these experiments are shown in Table 17.

TABLE 17. AMOUNTS OF BASIC GASEOUS CHEMICAL SUBSTANCES FOUND IN THE AIR ABOVE FECES STORED FOR 5 DAYS.

Substance	Amount, mg/100 g of feces		
	Maximum	Minimum	Average of 5 measurements
Ammonia and aliphatic amines	1.930	0.050	1.348
Aldehydes	Traces	Not found	
Ketones	0.352	Traces	0.159
Organic acids converted to acetic acid	1.490	0.116	0.855
Indole	0.07-0.037	Not found	0.021
Mercaptans and hydrogen sulfide	Traces	Not found	
Nitrogen oxides converted to N_2O_5	1.010	0.005	0.276
Carbon monoxide	0.063	0.002	0.023
Sulfide gas	0.330	0.0001	0.082
Hydrocarbons converted to C	1.975	0.285	0.901
Phenols	0.140	0.016	0.081

The Table shows that the following substances were found in the air above stored feces: ammonia and aliphatic amines, ketones, organic acids, indole, hydrogen sulfide with mercaptans, nitrogen oxides, carbon monoxide,

phenols and hydrocarbons, i.e., the same chemical substances that were found in the air above freshly excreted feces. In addition to the chemical substances listed above, the gaseous products from feces stored for 5 days always included sulfide gas.

It is also apparent from the Table that the amount of chemical substances in the air above feces stored for 5 days varies within considerable limits, which can be explained by peculiarities in the eating regimen of each "donor", the nature of the microflora, and other reasons.

A comparison of the average amounts of certain gaseous substances observed in the air above fresh feces and those that had been stored for 5 days shows that in the latter case the emission into the air of ammonia and aliphatic amines increased more than 70 times, the organic acids by more than 3.3 times, nitrogen oxides, 4.5 times, and phenols by about 9 times (Table 18). The content of organic substances (hydrocarbons) in the air above fresh feces and those that had been stored showed practically no variation, and the amount of carbon monoxide even decreased slightly. /63

TABLE 18. AVERAGE AMOUNTS OF CERTAIN GASEOUS CHEMICAL SUBSTANCES GIVEN OFF BY FRESH AND STORED FECES.

Substance	Amount, mg/100 g of feces		Change
	Fresh	Stored 5 days	
Ammonia and aliphatic amines	0.019	1.348	+70.9
Organic acids converted to acetic acid	0.259	0.855	+3.3
Nitrogen oxides converted to N_2O_5	0.061	0.276	+4.5
Hydrocarbons converted to C	0.802	0.901	+1.1
Carbon monoxide	0.122	0.023	-5.3
Phenols	0.009	0.081	+9.0

The differences found in the quantitative and qualitative characteristics of the gaseous products from fresh and stored feces must reflect the nature of their biological effect.

We assigned V. I. Mikhaylov and L. T. Poddubnaya to conduct a special investigation to examine the features of the combined action of the gaseous toxic substances given off by human feces stored in hermetically sealed glass desiccators for 5 days.

The methodology of these experiments was the same as that for the experiments in studying the features of the biological effect of the gaseous products from fresh feces. The biological effect was measured with the same biochemical and physiological indicators that were used in the preceding experiments.

The contents of chemical substances (in mg/liter) in the gaseous products given off into the chamber are listed below:

Ammonia and aliphatic amines	0.0032
Aldehydes	Not found
Acetone	0.001
Organic acids converted to acetic acid	0.018
Indole	0.00003
Mercaptans and hydrogen sulfide	Traces
Nitrogen oxides converted to N_2O_5	Traces
Carbon monoxide	0.018
Sulfide gas	0.001
Hydrocarbons converted to C	0.18
Phenols	0.0006
Carbon dioxide	1.20

Admission of this complex of gaseous substances into the experimental chamber produced phenomena in the animals that were characteristic of the action of irritant gases. These reactions died down only toward the end of the second hour of exposure. The animals calmed down and their breathing became uniform. /64

After terminating exposure, the animals manifested an increase in the time of the latent period of the flexor reflex in response to a single pulse of electric current (Figure 11), a statistically significant increase in the HbCO in the blood was observed (Figure 12), and there was a definite tendency toward an increase in the amount of acetylcholine and a definite drop in cholinesterase activity in the blood (Figure 13).

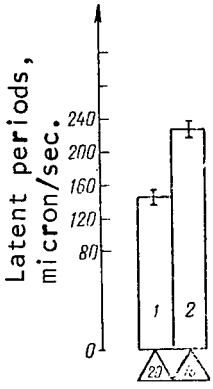

Figure 11. Effect of Toxic Substances Given Off by Stored Feces on the Value of the Latent Period of the Flexor Reflex in White Mice. Symbols same as in Figure 4.

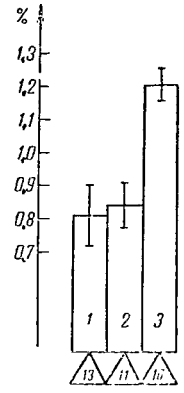

Figure 12. Effect of Toxic Substances Given Off by Stored Feces on the Carboxyhemoglobin Content in the Blood of White Mice. Symbols same as in Figure 7.

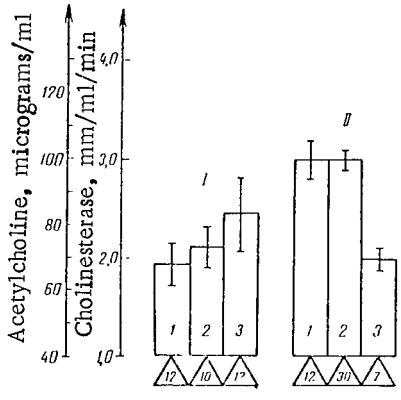

Figure 13. Effect of Toxic Substances Given Off by Stored Feces on the Content of Acetylcholine (I) and Cholinesterase Activity (II) in the Blood of White Mice. Symbols same as in Figure 7.

All of the changes just mentioned, in their degree and direction, are practically the same as those disturbances that were noted in animals subjected to the effect of gaseous products given off by fresh feces. Therefore, these experiments did not permit us to draw any conclusions as to whether the total biological effect of the gaseous products given off by stored feces exceeds the toxic effect of the mixture of toxic chemical substances that enter the air from fresh feces. /65

However, the data that were obtained do allow us to conclude that the toxic substances contained in the gaseous products given off by stored feces are far from being harmless as far as their effect on the organism is concerned, since they can cause not only an irritant effect but also lead to the development of generally toxic symptoms.

The toxicological danger of the chemical substances given off by feces requires the development of a series of measures to limit the possibility of entry of harmful additives to the atmosphere of hermetically sealed environments.

Among these measures, a great deal of emphasis must be placed on the discovery of chemical substances whose use can suppress the processes of decay and fermentation of excreta with the participation of microorganisms and at the same time can considerably reduce the liberation into the air of gaseous foul-smelling toxic compounds.

At the present time, a great many chemical substances are used for the /66
disinfection of human wastes, including fecal matter: chlorine preparations (bleaching powder, calcium hypochlorite, chloramine, etc.), phenol preparations (cresol, lysol, phenol and their derivatives, etc.), and other substances (Rogatina, 1967).

However, not all of them can be used for the preservation of feces stored in special containers in hermetically sealed locations, because the majority have a sharp odor, considerable toxicity, and can corrode metals. Therefore, work is currently under way in an effort to find chemical preservatives for feces which would be free of the above-mentioned defects but would have a high antimicrobial activity. *In vitro* studies conducted by L. N. Rogatina (1967) have shown that such properties are possessed by silver fluoride, silver nitrate, silver sulfate, copper salt "B", copper sulfate, iodine crystals, "feziomon", catamine "K", and FTA.

Of these substances, the most effective are the salts of silver and copper. Preservation of feces by preparations of silver (fluoride, nitrate and sulfate) and copper is achieved at a rate of 5-8 g/100 g of feces. Copper sulfate was effective at 30 g/100 g of feces. These preparations sharply reduced the specific odor of feces (deodorizing effect), but did not remove the odor caused by the liberation of gaseous products in the decomposition of organic substances contained in the feces.

One of the preparations developed by Rogatina (copper salt "B") was used by us to study the effect of chemical preservatives on the liberation of /67

certain gaseous toxic substances from feces stored for 5 days. These studies were conducted by us in conjunction with V. I. Mikhaylov, L. T. Poddubnaya and L. N. Rogatine (Kustov et al., 1967).

In these experiments, preservation of feces was accomplished by scattering a uniform layer of copper salt "B" over the feces at the rate of 8-10 grams of salt per 100 grams of excreta.

The preservative effect of salt "B" was evaluated on the basis of the results of microbiological analyses conducted by L. N. Rogatina. At the same time, we determined the content of gaseous chemical substances in the air above the feces, treated with preservative and stored without addition of copper salt "B".

The tests that were performed showed that while the number of microorganisms vegetating on the excreta increased markedly (especially the aerobic ones) during storage of feces without a preservative, when salt "B" was added the microbial growth on the feces was noticeably less. The antibacterial effect was practically 100% (99.9%) (Table 19).

TABLE 19. EFFECT OF COPPER SALT "B" ON MICROBIAL GROWTH ON FECES STORED FOR 5 DAYS (AVERAGE DATA FROM 5 EXPERIMENTS).

Feces	No. of microorganisms/1 gram of feces			
	Aerobes		Anaerobes	
	at start of test	at end of test	at start of test	at end of test
Without preservation	$5,6 \cdot 10^7 \pm 2,8 \cdot 10^7$	$35 \cdot 10^7 \pm 15,9 \cdot 10^7$	$1,8 \cdot 10^8 \pm 1,1 \cdot 10^8$	$21 \cdot 10^8 \pm 17 \cdot 10^8$
With preservation (copper salt "B")	$2,6 \cdot 10^8 \pm 1,1 \cdot 10^8$	$114 \pm 21,5$	$45 \cdot 10^7 \pm 17 \cdot 10^7$	$0,8 \pm 0,02$

Commas indicate decimal points.

The determination of the toxic substances in the air above stored feces showed that copper salt "B" reduced the liberation of ammonia and aliphatic amines from the feces by about 4 times, nitrogen oxides by 10 times, and organic compounds (hydrocarbons) by about 1.5 times. Indole and skatole, hydrogen sulfide and mercaptans, aldehydes and sulfide gas were more rarely

found in the air above "preserved" feces than in the background experiments (Table 20). Attention is called to the considerable content of carbon monoxide in the air above feces preserved with copper salt "B" (an increase relative to the background experiments of about 10 times).

The results of these tests allow us to venture the opinion that the microbial decomposition of feces liberates into the air mainly these substances whose content is reduced when a chemical preservative is used. The most important of these are: ammonia and aliphatic amines, nitrogen oxides, organic substances, and indole, skatole, aldehydes, hydrogen sulfide with mercaptans and sulfide gas. The increased liberation of carbon monoxide from preserved feces is evidently due to the fact that in the presence of copper salt "B" there is a more energetic oxidation of organic substances (including the porphyrinic compounds) entering into the composition of feces. /69

Hygienic Characteristics of Gaseous Chemical Substances Contained in Intestinal Gases

The formation of intestinal gases occurs in the process of the enzymatic and bacterial decomposition of the organic compounds entering into the composition of the contents of the large intestine. A certain amount of gas enters the intestine when air is swallowed during eating and breathing, as well as through the diffusion of certain gases from the blood.

Among the sources of intestinal gases, Hibbard (1936) lists the following: breakdown of intestinal contents (chemical and enzymatic decomposition, bacterial fermentation and putrefication); diffusion of gases from the blood; passage of atmospheric air through the intestine. This author mentions that it is primarily nitrogen diffusion that takes place from the blood into the gastrointestinal tract. The entrance of atmospheric air into the stomach accounts for about 50 ml of its gas content. The gas in the stomach consists of 80% nitrogen and 20% oxygen. The oxygen is absorbed to a certain degree, but the nitrogen passes on into the intestine (Kanter, 1919). According to other data, stomach gas consists of 15-16% oxygen, 5-9% carbon dioxide and 80-75% nitrogen (Davenport, 1966). The gastrointestinal tract in man usually contains 150 ml of gas. There is practically none in the small intestine, and about 100 ml in the large intestine (Davenport, 1966).

TABLE 20. EFFECT OF COPPER SALT "B" ON THE INTENSITY OF LIBERATION OF CERTAIN GASEOUS TOXIC SUBSTANCES FROM FECES STORED FOR 5 DAYS.

Experiment	Ammonia and aliphatic amines	Mercaptans and hydrogen sulfide	Phenols	Indole and Skatole	Organic acids converted to acetic acid	Sulfide gas	Ketones	Aldehyde	Nitrogen oxides converted to N_2O_5	Carbon monoxide	Hydrocarbons converted to C
	Amount, mg/100 g of feces										
Without preservative											
1	0,006	Traces	0,018	0,009	0,184	0,041	0		0,876	0	0,945
2	0,238	0	0,011	0,005	0,136	0	0,043		0,012	0	0,315
3	0,375	Traces	0,011	0	0,078	0,006	0,008	Traces	0,074	0,063	0,630
4	—	0	0,010	0	0,015	0	0,044		0,005	0	0,552
5	Traces	Traces	0,002	0	0,120	0,005	0		—	0,051	0,964
M~	0,167	—	0,010	—	0,107	0,010	0,019		0,242	0,023	0,881
With copper salt "B"											
1	0,197	0	0,020	0,006	0,069	0	0,0280	0	0,038	0,208	0
2	0,009	0	0,007	0	0,428	0,012	0,009	0	0,007	0,423	0,191
3	0	Traces	0,035	0	0,100	0,046	0,006	0	0,016	0,243	0,152
4	0	»	—	0	0,130	0	0,013	0	0,033	—	1,890
5	0,019	0	0,004	0	0,078	0	0	0	0,023	0,207	0,630
M~	0,045	—	0,017	—	0,161	—	0,011		0,023	0,270	0,578

Commas indicate decimal points.

The composition and quantity of the intestinal gases in different individuals undergo considerable variations depending on the amount and nature of the food eaten, peculiarities of the functioning of the gastrointestinal tract, the nature of the microflora in the intestine, and a number of other factors. The effect of food on the intensity of the formation of intestinal gases was demonstrated by Murphy (1964); if the intensity of formation of intestinal gases in healthy individuals is 20-50 ml/hr, it increases by a factor of 10 to 20 about 3 to 4 hours after eating beans. This increase in /70
the production of intestinal gases according to the author's data, comes mainly from carbon dioxide and hydrogen. Data on the effect of diet and eating regimen on the formation of intestinal gases are contained in the paper by Hedin and Adachi (1962).

At the present time, it is assumed that in the intestines of a practically healthy individual about 800-1,000 ml of gas daily is formed (Fries, 1906; Kirk, 1949). The rate of gas formation varies from 0.36 to 3.97 ml/min and amounts to about 1.47 ml/min on the average (Kirk, 1949).

The volume of gas expelled at any one time varies from 50 to 500 ml (the latter occurs when intestinal gases have been held back for many hours) and amounts to 100 ml on the average (Fries, 1906).

The chemical composition of intestinal gases is rather complex (Table 21). The intestinal gases contain small quantities of the same gaseous chemical substances formed in the air above fresh feces. In particular, these substances include indole, skatole, hydrogen sulfide with mercaptans, sulfide gas, volatile organic acids and other gaseous foul-smelling chemical compounds (Tarkhanov, 1888; Korenchevskiy, 1909; Slager, 1962).

Most of the total volume of intestinal gases consists of oxygen, nitrogen, carbon dioxide, hydrogen and methane (Murphy, 1964; Slote, 1965).

According to Fries' data (1906), 1,254 ml of intestinal gases contain approximately 28.6 ml of CO_2, 201 ml of methane and 35.7 ml of hydrogen.

TABLE 21. COMPOSITION OF HUMAN INTESTINAL GASES (SLOTE, 1965).

Substance	Concentration, mole %	Maximum allowance concentration, mole %	Substance	Concentration, mole %	Maximum allowable concentration, mole, %
Carbon dioxide	5.9-3.8	1*	Nitrogen	10-87.7	--
Hydrogen	0-54	3**	Oxygen	0-10.3	--
Hydrogen sulfide	0-6.4 10^{-4}	2 10^{-3}	Methane	0-55	5.3**

* For Pl.Pl. converted to 30 days.
** Minimum concentration for explosion hazard.

Best and Taylor (1958), using their own experimental material, conducted some calculations according to which 1,000 ml of intestinal gases can /71
contain approximately 230 ml of carbon dioxide, 210 ml of nitrogen, 80 ml of oxygen and 480 ml of methane and hydrogen. Somewhat different figures are given by Davenport (1966). In his work on the physiology of the gastrointestinal tract, he states that the gas in the large intestine does not contain oxygen, but consists of 50% nitrogen and 40% carbon dioxide. The remaining 10% consists of methane, hydrogen, hydrogen sulfide and other products of the fermentative activity of bacteria. This author explains the considerable quantity of carbon dioxide in the large intestine by the interaction of bicarbonates in the intestinal contents with acids produced by microbial flora.

Clemedson (1959), guided by the data of Kirk (1949) on the composition of intestinal gases (given below), conducted a hygienic estimate of their basic components:

Gas	Concentration, %	Gas	Concentration, %
Hydrogen	0-37.2	Carbon dioxide	5.9-24.7
Oxygen	0-10.3	Hydrogen sulfide	0-0.00064
Nitrogen	24.7-87.7	Methane	0-34.1

In particular, he determined the time during which the contents of the principal components of intestinal gases can reach hygienically significant values in the atmosphere of a standard cabin (Table 22).

TABLE 22. TIME REQUIRED FOR INTESTINAL GASES TO ACCUMULATE TO MAXIMUM PERMISSIBLE CONCENTRATION DURING STAY OF ONE PERSON IN A HERMETICALLY SEALED STANDARD CABIN (CLEMEDSON, 1959).

Gas	Concentration, ppm	Concentration, mg/l*	Time, days	Effect
Hydrogen sulfide	20	0.029	148	Irritation of eyes, nausea
Hydrogen			44	Explosion
Methane			59	Explosion
Carbon dioxide	5,000	9.0(~0.5%)	10	Slight hyperventilation
Carbon dioxide	30,000	54.0(~3.0%)	60	Panting

*Our conversion--(V.K., L.T.)

On the basis of the results of his calculations, Clemedson came to the conclusion that hydrogen sulfide (in the concentrations in which it occurs in intestinal gases) can produce hygienically significant results only after 148 /72 days. As far as carbon dioxide is concerned, its possible concentration in the atmosphere of a standard cabin can lead to changes in respiration after 10-60 days. The author considers these changes to be insignificant, however.

It is striking that the possible accumulation of hydrogen and methane in the cabin atmosphere can reach such concentrations that there is a risk of an explosion. The maximum permissible concentrations of these gases are 4.1 and 5.3%, respectively. They are based, of course, on the explosive properties of methane and hydrogen, since neither has any biological effect in the concentrations given (Calloway, cited by Murphy, 1964).

A study of the toxicity of intestinal gases has been conducted by several authors in connection with the problem of autointoxication. In special experiments on cats, it was shown that a critical role in the toxic effect of intestinal gases is played by hydrogen sulfide. Similar effects were obtained by injection of solutions of intestinal gases and pure hydrogen sulfide (Teschendorf, 1922, 1922a; McSver, Redfield and Benedict, 1926; Hibbard, 1936). However, the calculations of Clemedson given above show that for the conditions of hermetically sealed cabins, the entire complex of chemical substances making up intestinal gases are of hygienic significance.

Characteristics of Gaseous Chemical Substances Given Off by Man in the Expired Air

The chemical composition of the air expired by man is very complex. It contains 75.7% nitrogen, 15.3% oxygen, 5.1% water vapor and 3.95% carbon dioxide, (cf. Clemedson, 1959). In addition to the above, expired air always contains chemical compounds that are formed in the organism in the course of normal metabolism and are excreted through the lungs. Expired air contains some of the components of intestinal gases, which diffuse into the blood from the intestine [for example, hydrogen, methane, ethane (Calloway, cited by Murphy, 1964)] as well as volatile compounds contained in the secretions of the salivary glands or formed in the oral cavity as a result of microbial decomposition of saliva and food residue, as well as chemical substances polluting the atmosphere of cities and populated areas.

At the present time, the saliva and expired air of practically healthy individuals have been found to contain 20 chemical compounds, while samples taken from the oral cavity have been found to contain about 100 different chemical substances (Weber, 1967).

However, the different origin of the same components of expired air often complicates the solution of the problem of whether a given substance is characteristic of expired air or whether its presence is accidental. /73

A constant component of expired air is carbon monoxide. Its presence in expired air from man was first pointed out by Jeckson (1887, cited by Peters, 1906), who attempted to use this to explain its harmful effect on the organism. As of the present day, the endogenic formation of CO and its excretion in the expired air have been demonstrated by many investigators, whose tests were conducted with the use of diverse highly-sensitive methods (Tiunov and Kustov, 1966).

The content of carbon monoxide in the expired air from healthy non-smokers, according to the data of various authors, is 0.0028mg/liter (Sjoestrand, 1949), 0.016 mg/liter (Bogatkov et al., 1961), and 0.011 mg/liter (Kustov et al., 1962). The rate of excretion of CO from the organism usually does not exceed 0.5-1.05 cm^3/hr (Sjoestrand, 1951) and consists on the average

of 10 cm^3 daily (Still, 1960; Slager, 1962). The accumulation of CO in the atmosphere of hermetically sealed areas occurs quite rapidly. According to the data of P. I. Bogatkov, Yu. G. Nefedov and M. N. Poletayev (1961), the air of a hermetically sealed chamber with a volume of 24 m^3 (with three subjects inside) showed a carbon monoxide content of 0.023-0.027 mg/liter by the 9th-10th day of the experiment. In the pumping chamber of the insulating apparatus the CO concentration can amount to 0.02-0.04 mg/liter even after only 15-20 min of the experiment, (Gorodinskiy et al., 1967), which somewhat increases its maximum allowable limit as calculated from an 8-hour exposure (0.02 mg/liter, cf. State Standard 245-63).

Another constant component of expired air is ammonia (and its compounds) (Clemedson, 1959; Still, 1960; Slager, 1962; Slote, 1965 et al.).

We have discussed the formation and excretion of ammonia from the organism in Chapter II of this book.

Its content in the expired air from practically healthy subjects, according to the data of various authors, is from 0.000008 to 0.000024 mg/liter (0.000011 mg/liter on the average; Alpatov, 1962), 0.517 ± 0.321 μg NH_3 - N/liter (Mueller-Beissenhirtz and Keller, 1965), and the daily excretion of ammonia is from 0.02 g (Zhandr, 1897) to 0.08- 0.4 (Gorodinskiy et al., 1967).

The ammonia level in the expired air from man varies a great deal depending on the nature of the diet and its content in the blood and inspired air. A considerable influence on the ammonia concentration is exerted by the stata of the oral cavity, its content in the saliva, etc.[9]

Thus, for example, if the ammonia content in the oral cavity is 2 mg/liter /74
before brushing the teeth with "Lesnaya" toothpaste, its presence in the oral cavity cannot be detected after cleaning the teeth (Fedorov, 1967). According to the data of Mueller-Beissenhirtz and Keller, 1965), the ammonia concentration in the expired air fell from 0.725 to 0.200 μg of NH_3 - N/liter after rinsing the oral cavity.

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[9] The amount of ammonia in the saliva is 2-10mg% (Mueller-Beissenhirtz and Keller, 1965).

Another constant component of expired air from practically healthy individuals is acetone (Shur, Lyashko, 1952; Weber, 1967; Heine, 1967; Hubbard, 1920; Briggs, Schaffer, 1921; Slager, 1962; Stolmann, 1965; Larsson, 1965 et al.). Its content in the expired air in healthy individuals varies from trace amounts to 0.144 mg/liter. When fat is used (up to 200 g of fat), the acetone concentration in the expired air reaches 2.8 mg/liter (Lyashko, 1952). Data on the acetone content in (mg/liter in the expired air of healthy individuals on the basis of the results of the studies of various authors are as follows: 0.34 ± 0.02 (Handerson, Karger and Wrenshall, 1952); 0.063-144 (Glaubitt and Rausch-Strooman, 1959); 0.2 (Glaubitt, 1961); 0.97 ± 0.07 (Levey, Balchum, Medrano and Jung, 1964); 0.99-0.59 (Eriksen and Kulkarni, 1963). The daily amount of acetone given off by an individual in the expired air is 0.03--0.08 g (Makeyev, Gulevich and Broude, 1947).

A high acetone content in the expired air is observed with certain liver ailments, sugar diabetes, cardiovascular pathology in the state of decompensation, etc. In these cases, the acetone level in the expired air can reach 21.3 mg/liter (Shur, 1939). For details on the effect of various factors on the intensity of acetone excretion, see Chapter III.

Expired air has been found to contain methane, small amounts of acetoacetic acid, volatile fatty acids, methyl alcohol, ethanol, methylethyl ketone, and acetonitrile (Clemedson, 1959; Slager, 1962; Eriksen, Kulkarni, 1963; Freund, 1965; Larsson, 1965; Weber, 1967, etc.). The amounts of these compounds in the expired air are relatively small. Thus, the methane content in the expired air from healthy subjects varies from 1 to 99 ppt (Levey and Balchum, 1963).

The secretions of the oral cavity contain ammonia, hydrogen sulfide (Fedorov, 1967), acetone, methane, ethanol (Larsson, 1965) and other volatile chemical compounds that give off an unpleasant odor.

One source of bad breath is the certain components of saliva that have in their composition sulfhydryl groups or other substances with sulfur in them, compounds containing amines and ammonia, as well as the products of putrescent decay in the nasopharynx and carious teeth (Fedorov, 1967). Small

amounts of these gaseous products formed in the oral cavity are included, as a rule, in the composition of the expired air.

A number of the chemical substances that enter into the composition of /75
expired air exhibit pronounced toxicity, and their accumulation in the atmosphere of hermetically sealed cabins of small volume constitutes a definite danger for a healthy individual.

To evaluate the degree of this danger, we worked in conjunction with V. I. Mikhaylov and L. T. Poddubnya to determine the content of individual toxic substances in the expired air from human subjects.

Healthy men 20-22 years old took part in the test. A diagram of the apparatus used to collect samples of expired air for subsequent chemical analysis is shown in Figure 14.

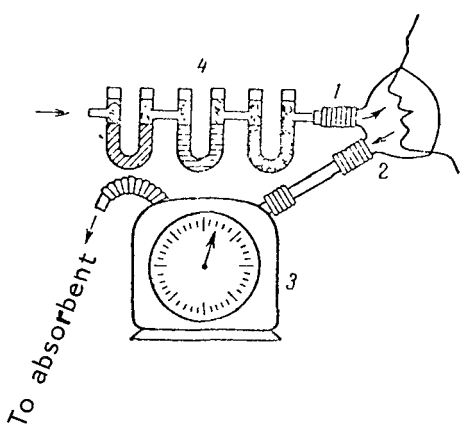

Figure 14. Diagram of Apparatus for Collecting Expired Air From a Human Subject. 1, Intake valve; 2, Exhaust valve; 3, Hydroscope; 4, System for purifying room air.

The tests which we conducted showed (Table 23) that the expired air of practically healthy persons always contains ammonia and its compounds, aldehydes, ketones, organic acids, carbon monoxide, carbon dioxide, water vapor and hydrocarbons. Of these, 33% are saturated hydrocarbons, 5.4% are unsaturated and 1.2% are aromatic. The concentrations of these chemical compounds in the expired air from the subjects was highly variable, evidently due to individual peculiarities of metabolism, content of various compounds in the oral cavity, varying nature of microflora and other causes.

Therefore, in calculating the time required for attaining the limit of the allowable content of some of the substances listed in Table 23 in the

atmosphere of a standard cabin, we shall proceed on the basis of the values of their mean concentration in the expired air.[10]

TABLE 23. AMOUNTS OF SEVERAL TOXIC SUBSTANCES IN THE AIR EXPIRED BY HUMAN BEINGS.

Substance	No. of determinations	Concentration, mg/liter		
		Minimal	Maximal	Average
Ammonia and its compounds	27	Traces	0.005	0.0022
Aldehydes	13	"	0.0015	
Acetone (ketones)	49	"	0.0025	0.0002
Organic acids converted to acetic acid	14	0.007	0.013	0.02
Carbon monoxide (non-smokers)	22	0.003	0.017	0.011
Hydrocarbons converted to C	17	Traces	0.051	0.036
saturated (33%)	--	---	---	---
unsaturated (5.4%)	--	---	---	---
aromatic (1.2%)	--	---	---	---
Carbon dioxide (in %)	7	2.0	3.9	3
Water vapor (in %)	7	20	50	38

The values of these time segments are shown in Table 24. /76

TABLE 24. POSSIBLE TIME REQUIRED TO REACH LIMIT OF ALLOWABLE CONCENTRATION OF CERTAIN COMPONENTS OF EXPIRED AIR UNDER THE CONDITIONS OF A STANDARD CABIN.

Substance	Average concentration in expired air, mg/liter	Time to reach limit of allowable concentration, in hours	Limit of allowable concentration (State Standard 245-63) mg/liter
Ammonia and its compounds	0.0022	~3	0.02
Acetone	0.0002	~14 days	0.2
Organic acids converted to acetic acid	0.013	~1.33	0.005
Carbon monoxide	0.011	~7	0.3
Hydrocarbons converted to C	0.036	~30	0.3

[10] The calculation was performed with consideration of the value of the hourly air exchange in a healthy individual.

It is clear from the Table that the extreme attainable level based on an 8-hour exposure in the atmosphere of a standard cabin (due solely to expired air) can be reached for carbon monoxide in about 7 hours, for ammonia and its compounds in 3 hours, for organic acids in about 1 hour and 20 minutes, for hydrocarbons in about 30 hours, and for acetone in only 14 hours.

All of the above indicates that the toxic components of expired air can play a considerable role in the formation of the atmospheres of hermetically sealed cabins of small volume.

Having determined the hygienically significant concentrations of certain toxic substances in the composition of the expired air, we decided to conduct a special study to determine the nature of its biological action. This type of investigation was also dictated by the extremely contradictory data on this subject (see Chapter I).

In the experiments which we conducted in conjunction with M. M. Korotayev, V. I. Mihaylov, G. I. Meleshko and Ye. Ya. Shepelev (Kortayev, Kustov et al., 1967), we studied the effect of a complex of toxic substances which enter into the composition of the expired air of man on the photosynthetic activity of Chlorella (chlorella pyrenoidosa), cultured in a Tamiy medium in a special photosynthetic gas exchanger.

It was found in these experiments that the expired air introduced /77
into the air space of the gas exchanger caused noticeable suppression of the photosynthesis of chlorella, as measured by the change in the rate of CO_2 absorption in percent of the control experiment value (Table 25).

Later on, we continued our study of the biological effect of air expired by man in experiments on white male mice weighing 20-22 g.[11] The following is a list of the chemicals composing the expired air of a human subject which /78
was collected in Douglas bags and then circulated for two hours through a chamber containing the experimental animals.

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[11] We conducted the studies in conjunction with V. I. Mikhaylov and L. T. Poddubnya (unpublished data).

TABLE 25. CHANGE IN RATE OF PHOTOSYNTHESIS OF CHLORELLA UNDER THE INFLUENCE OF AIR EXPIRED BY A HUMAN BEING.

Duration of experiment in hours	Change in density of suspension during the experiment, million k liter/hr	Change in rate of absorption of CO_2, % of control	Duration of experiment in hours	Change in density of suspension during the experiment, million k liter/hr	Change in rate of absorption of CO_2, % of control
6	940—1,228	-2	3	1,700—1,920	-6
4	900—1,168	-12	11	1,080—2,052	-19
2	1,300—1,407	-20			

In view of the reduced oxygen content in the gas mixture obtained (16.5-17%), the latter was added to the composition of the expired air to give a concentration of 20.5%.

Ammonia and aliphatic amines..... 0.0011 mg/L
Acetone..... 0.0008 mg/L
Organic acids converted to acetic acid..... 0.057 mg/L
Carbon monoxide..... 0.003 mg/L
Carbon dioxide..... 3.3 vol.%
Oxygen (after enrichment of mixture).....20.5 vol.%
Total oxidizability, mg O_2..... 0.106

To exclude the toxic effect of CO_2 contained in significant amounts in expired air (about 3.3%), we set up a series of control experiments to study the effect on the animals of a nitrogen-oxygen mixture with an increased concentration of carbon dioxide (nitrogen = 76.5%, oxygen = 20.5%, CO_2 = 3%). /79

The results of these experiments showed that while this gas mixture did not produce visible changes in the behavior of the animals, after a 2-hour exposure to expired air enriched with oxygen the experimental mice became sluggish and inert.

After the conclusion of the experiment, the content of carboxyhemoglobin and acetylcholine in the blood of the experimental animals was significantly higher than in the control group of mice (Figure 15). Similar changes were seen with respect to the catalase and cholinesterase activity of the blood (Figure 16).

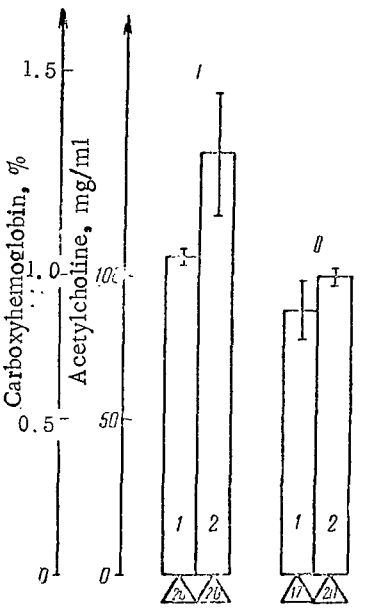

Figure 15. Effect of Toxic Substances Contained in Expired Air on the Level of Carboxyhemoglobin (I) and the Content of Acetylcholine (II) in the Blood of White Mice. Symbols same as in Figure 4.

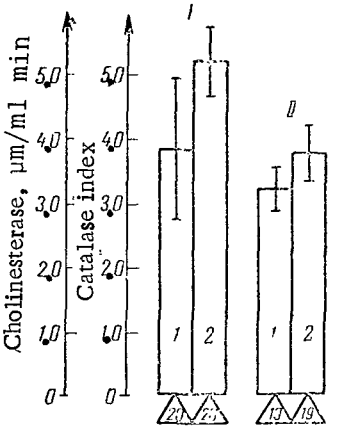

Figure 16. Effect of Toxic Substances Contained in Expired Air on the Cholinesterase (I) and Catalase (II) Activity of the Blood of White Mice. Symbols same as Figure 4.

The physiological significance of the changes we observed in the state of the experimental animals is apparently not great. However, their occurence allows us to conclude that the complex of toxic substances contained in the expired air of practically healthy individuals is not a matter of indifference to the organism.

The Role of the Skin in the Excretion of the Metabolic Products of the Organism. Composition and Hygienic Significance of Certain Gaseous Substances Accumulated in the Space Beneath the Clothing.

The list of metabolic products excreted by man includes chemical substances that are liberated into the surrounding medium with the secretions of the cutaneous sweat and sebaceous glands as well as in the course of cutaneous respiration. Some of these substances are far from harmless to the organism, as shown in a series of papers by Soviet and foreign investigators (cf. Petrun', 1960).

The results of the investigations indicated that the gas exchange through the skin and its secretory function play an important role in the vital activity of the organism. However, the limited possibilities of analytical chemistry until recently have not allowed us to perform the chemical identification of the majority of these toxic substances. Therefore, the reason for the death of animals in which the respiratory and secretory functions of the skin were interrupted was made the subject of attempts by investigators to link it with asphyxiation (Gerlach, 1851) or with a disturbance of the thermoregulatory function of the skin (Lashkevich, 1868; Martens, 1900), or with the accumulation in the organism of some kind of volatile hypothetical substance (Zhandr, 1889), or with the entrapment in the organism of ammonia and other nitrogenous compounds (Sokolov, 1874).

At the present time, with the aid of modern methods of chemical analysis (chromatography, spectrophotometry, spectrometry, etc.) we have succeeded in /80
determining the chemical composition of the secretion of the sweat and sebaceous glands in the skin, as well as the products excreted from the organism through the skin.

We know that there is excretion of carbon dioxide and absorption of oxygen through human skin as the result of the difference in the partial pressures of oxygen and CO_2 in the blood and the surrounding air. As the gaseous composition of the air changes, the exchange of CO_2 and oxygen through the skin also varies. With an increased concentration of CO_2 there is absorption of carbon dioxide by the skin and excretion into the atmosphere of large amounts of oxygen. With an increased amount of oxygen in the surrounding medium, its penetration into the blood through the skin increases markedly; this is especially noticeable under conditions of high temperature of the surrounding medium (Petrun', 1960).

In the process of cutaneous respiration, in addition to carbon dioxide and oxygen, the gaseous products usually excreted through the lungs (mainly ammonia, acetone, and other substances) may also be liberated into the surrounding medium (Slote, 1965).

The secretory function of the skin is accomplished by the sweat and sebaceous glands. The daily output of sweat varies from 400-600 ml; under certain conditions, sweat secretion can rise to 10 liter and more (Kartamyshev, 1953; Kuno, 1961).

The chemical composition of sweat depends on the features of metabolism, the state of the neuro-psychological sphere of the individual, the temperature of the surrounding medium, the nature and intensity of muscular activity, etc. The limitations to the methods of collecting sweat from the surface of the body may to a certain degree distort the true composition of the secretion of the sweat glands. If sweat is collected during the initial period of sweating, it will contain not only the substances that enter into the composition of the secretion of the sweat glands, but also chemical compounds usually located on the surface of the skin (secretion of the sebaceous glands, products of decomposition of this secretion, dirt on the skin, etc.). Only the composition of the sweat collected during profuse sweating can really correspond to the secretion of the cutaneous sweat glands.

The composition of the secretion of the sweat glands contains small amounts of a number of organic and inorganic compounds (after Clemedson, 1959):[12]

Water, %	99.2-99.7	Iron, mg%	0.1-0.2
Solid substances, %	0.26-0.78	Iodine, fluorine and bromine	Traces
Organic solid substances, %	0.03-0.29	Lactic acid, mEq/l	4-40
Ash, %	0.14-0.57	Glucose, mg%	0.1-9
Sodium chloride, mEq/l	5-148	Nitrogen, mg%	23-140
Potassium, mEq/l	1-15	Urea nitrogen, mg%	12-39
Calcium, mEq/l	1-8	Ammonia, mg%	5-9
Magnesium, mg%	0.4-0.4	Creatinine, mg%	0.1-1.3
Copper, mg%	0.4-7.5	Uric acid, mg%	0-15
Manganese, mg%	3.2-7.4	Amino acid nitrogen	Traces
Sulfates, mg%	4-17	Phenol	Traces
		Histamine	Traces

/81

In addition to the substances listed in the Table, the secretion of the sweat glands has also been found to contain arginine, histidine, lysine,

[12] The chemical composition of human sweat is also given in works by Ya. Kuno (1961) and S. Robinson and A. Robinson, 1954).

phenylalanine, tryptophan, serine, glutamic acid, aspartic acid, proline, methionine and cystine (Ito and Makayama, 1952, cited in Yas and Kuno, 1961). I. Kral and A. Zenisek (1958) found the histidine derivative, urocanic acid in the amount of 10 mg% in the composition of sweat. Sweat also contains small amounts of volatile fatty acids: formic, acetic, butyric, propionic (Renk, 1886) and sulfuric acid esters, lipids, vitamins, acetylcholine and other choline-like substances (Kuno, 1961; Clemedson, 1959).

The combination of chemical substances included in sweat manifests a pronounced biological activity. The introduction of human sweat into the blood of experimental animals causes a number of serious disturbances in the organism, which in many cases lead to a fatal outcome (Lacour, Cier, 1963).

The chemical composition of the secretion of the sebaceous glands has been studied in less detail than that of the sweat glands. Normally functioning sweat glands excrete in the course of a week about 100 g (and sometimes 200-300 g) of "cutaneous fat," containing olein, palmitin, the salts of fatty acids, proteins, extractives, etc. (Kartamyshev, 1953).

According to the data of Yu. V. Korolev (1961), the cutaneous fat of healthy individuals contains about 30% free fatty acids, 38% esters of fatty acids, 32% unsaponifiable substances (cholesterin, squalene, etc.). The free fatty acids (caproic, enanthic, etc.) amount to 5.5% of the weight of the cutaneous fat. There is about 24.3% higher fatty acids, 7.8% bound lower fatty acids, 31.7% higher fatty acids, and 2.5% cholesterin (by wt. of cutaneous fat). The secretion of the sebaceous glands in the skin also contains proteins, inorganic acids and their salts (Slote, 1965).

Pollution of the cutaneous covering is not only the result of the secretions of the sebaceous and sweat glands. The surface of the skin contains scaled-off epithelium, fallen hair and particles of feces and urine. Under the action of the oxygen in the air, and also as the result of the action of enzymes of microorganisms that grow on the skin, there is decomposition of the organic and inorganic substances contained in the above-mentioned sources of pollution. These processes considerably complicate the chemical /82

composition of the cutaneous pollutants, in which 271 chemical substances have already been found (Weber, 1967).

However, not all the substances that pollute the skin participate in the formation of the air medium in the space beneath the clothing and the atmosphere of hermetically sealed enclosures of small volume. The substances which are liberated into the surrounding medium from the cutaneous excreta consist mainly of volatile organic acids that cause the specific odor of sweat (Clemedson, 1959), CO_2, ammonia and other nitrogenous compounds (Il'inskiy, 1882) as well as methane and hydrogen (Slager, 1962).

In conjunction with G. M. Gorban, I. I. Kondrat'yeva and L. T. Poddubnaya we determined the intensity of excreation of several gaseous chemical substances that may appear in the space beneath the clothing.

For this purpose, we made a special hermetically sealed suit out of polyethylene sheeting. The subject donned this suit in a well-ventilated room and kept it on (together with a pair of undershorts) for 2-6 hours. The experiments were conducted at normal barometric pressure, at an air temperature of 19-20° with a relative humidity of 50-60%.

The movement of the air that was pumped into the suit by a blower following preliminary cleaning is shown in Figure 17. The air from the space beneath the suit, after passing through a system of absorbers filled with the proper substances, was returned again to the suit. The rate of movement of the air beneath the suit was set so that the organism was not overheated. For this purpose, the subject's temperature was taken orally every 30 minutes during the test. During the time of the experiment, its /83
value varied within the limits of 36.4-37.0° in different persons.

The content of chemical substances was determined in the air and condensate collected from beneath the suit (Table 26).

The data presented in the Table indicate that cutaneous excretion can contribute the following substances to the air of hermetically sealed environments: ammonia and its compounds, gaseous chemical substances from the ketone and aldehyde groups, volatile organic acids, mercaptans and hydrogen sulfide,

carbon monoxide, and certain organic compounds, determined as a whole on the basis of carbon.

TABLE 26. CONTENT OF CERTAIN GASEOUS CHEMICAL SUBSTANCES IN THE AIR AND CONDENSATE COLLECTED FROM THE SPACE BENEATH THE SUIT.

Substance	Number of determinations	Average amount of substance (in mg) in 2 hrs., of expt.	Content of substance in mg		Distribution %	
			In air	In condensate	Air	Condensate
Ammonia and its compounds	22	1,38	0,04	1,34	2,5	97,5
Aldehydes	20	0,08	0	0,08	0	100
Ketones	19	4,78	0,76	4,02	16	84
Organic acids converted to acetric acid.	20	1,86	0	1,86	0	100
Mercaptans and hydrogen sulfide	18	0,46	0,06	0,40	12	88
Carbon monoxide	19	0,023	0,023	0	100	0
Hydrocarbons converted to C	20	0,024	0,024	0	100	0

Commas indicate decimal points.

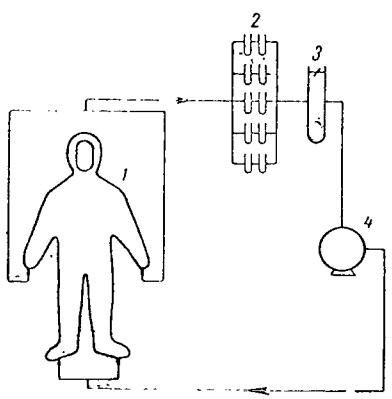

Figure 17. Diagram of Apparatus to Collect Samples of Air From Beneath a Space Suit. 1, Polyethylene suit; 2, System of chemical absorbents; 3, Rheometer; 4, Blower; Arrows: Direction of air flow.

Some of these compounds dissolve readily in water (ammonia and its compounds, aldehydes, ketones, organic acids, hydrogen, sulfide and mercaptans). Therefore, their content in the air in the space beneath the clothing is usually relatively small. Thus, for example, the amount of ammonia and its compounds in the space beneath the clothing is about 2.5% of its total volume; for ketones the figure is 16%, and for mercaptans and hydrogen sulfide it is 12%. However, this is by no means an indication that the role of water-soluble gaseous products of cutaneous excretion in the formation of the air medium of hermetically sealed cabins is small.

Dissolved in the water excreted in cutaneous perspiration, these substances are absorbed by the clothing and are given off constantly into the atmosphere of the cabin in the process of desorption. Therefore, their content in the composition of the cutaneous excretions, as well as the content of carbon monoxide and evidently methane too, must be taken into account in considering the total excretion of metabolic products into the atmosphere of a cabin of small volume.

CHAPTER V

COMPOSITION OF THE AIR IN A HERMETICALLY SEALED CHAMBER OCCUPIED BY HUMAN BEINGS

A considerable body of material has been accumulated, dealing with the /84
problems of the formation of the air environment in hermetically sealed chambers (Konecci, 1959; Schaefer, 1959; Siegel, 1960; Still, 1960; Thompson, 1960; Lambersten, 1961; Ramskill, 1961; Slager, 1962; Schulte, 1961, 1964; Schaefer, 1964; Slote, 1965 et al.).

The atmosphere in a spacecraft cabin consists either of nitrogen and oxygen, or of helium or oxygen. The cabin atmosphere also contains gaseous products given off by human beings, equipment, coverings and other sources (Burnazyan, Nefedov, Parin et al., 1967).

The most complete data on the composition of an atmosphere of this kind have been obtained in long-term experiments conducted in spacecraft simulators.

Thus, in a 56-day experiment with 4 volunteers in a spacecraft simulator, gas chromatography, infrared spectrometry and mass spectrometry were used by Adams et al., (1966) to detect 68 chemical substances whose concentrations were below the physiologically active level and which had no significant effect on the state of health or working ability of the individuals (Ulvedal and Roberts, 1966; Bartek, Ulvedal and Brown, 1966; Glatte and Giannetta, 1966).

In the atmosphere of the "Mercury" spacecraft, Toliver and Morris (1966) found 59 chemical substances (30-day experiment with one subject).

In a 30-day experiment with 3 subjects, Toliver et al. (1966) analyzed the gas content of the capsule. The systems for oxygen regeneration and removal of excess carbon dioxide and hydrogen were in operation. The subjects were forbidden to smoke. Using the latest techniques of analyzing the air, the authors found more than 60 compounds. These included mainly the following: oxygen, nitrogen, hydrogen, carbon dioxide, carbon monoxide, methane, /85
propane, n-butane, butane-1, n-pentane, hexane-1, trans-butane-2, cis-butane-2,

isopentane, 3-methyl pentane, 2,2-dimethyl butane, cyclohexane, transhexane, 1,1-dimethyl cyclohexane, ethyl-cyclohexane, ethylene, propylene, isobutylene, acetylene, isoprene, toluene, benzene, o-xylol, m-xylol, p-xylol, 1-chloropropane, trichloroethylene, ethylene dichloride, vinyl chloride, vinylidene chloride, methylene chloride, methyl chloroform, acetone, methyl-ethyl ketone, methyl-isopropyl ketone, methyl alcohol, ethyl alcohol, propyl alcohol, butyl alcohol, propionic acid, butyric acid, acetaldehyde, formaldehyde, ethyl formiate, ethyl acetate, n-propyl acetate, n-dioxane, freon-11, freon-114, hydrogen sulfide and SO_2.

Approximately 70 chemical substances were found in the atmosphere of a space capsule in the long-term experiments (17-30 days) conducted at the School of Aviation Medicine in San Antonio, Texas (McKee, Rhoades et al., 1963). The following were the principal substances found in the atmosphere of a "Mercury" capsule simulator: acetaldehyde, acetylene, acetone, ammonia, benzene, carbon monoxide, carbon tetrachloride, dimethylsulfide, ethanol, methanol, ethylene oxide, isoprene, isobutyryl aldehyde, methyl chloride, methyl-ethyl ketone, sulfur anhydride, toluene, dichloroethylene, trichloroethylene, and other compounds (cf. McKee et al., 1963; Slote, 1965 et al.).

In pointing out possible sources of harmful contaminants, the above authors list primarily plastics, life-sustaining systems and other cabin equipment, and finally, man himself.

Several investigators (in particular, Concle, Mabson et al., 1967) have made an attempt to determine the role of Man in the formation of the atmosphere of a spacecraft cabin simulator. In the course of a 27-day experiment, the authors identified and quantitatively evaluated 97 chemical compounds. A total of 21 substances were found after 2 subjects had lived in the chamber (14-27 day experiments). At this stage of the experiment, the air of the simulator contained the following compounds: propionic and valerianic acids, butyraldehyde, mesitylene, methyl-p-butyrate, butyl acetate, furan; 1,4-dimethoxybenzene, dimethyl furan, benzyl ether, pentafluoroethane, freon-113, skatole, decaline, isomers of decaline, methane, propyl mercaptan, ethane, methyl amine, ethylene and carbon monoxide. The amount of the latter in the

simulator at an air temperature of 21.1° and normal barometric pressure rose at the rate of 0.39 ml/man/hr. The rate of increase of methane reached 2.04 ml/man/hr. The data from this experiment clearly show the importance of human metabolic products in the contamination of the atmosphere of hermetically sealed chambers. However, studies in chambers filled with materials and /86
equipment make it difficult to determine the significance of the true "human contaminants," since the majority of products of human vital activity belong to the same chemical classes of compounds of which man is not the sole source. More indicative in this respect are the results of experiments conducted in ventilated smooth-walled chambers. In similar experiments, it was found that a practically healthy individual excretes 0.3 liters of methane daily. At this rate of excretion, the methane concentration in a hermetically sealed chamber can reach a level dangerous to life after 30-40 days of an experiment (Gorban' et al., 1964).

With carbon monoxide content in the expired air of 0.016-0.038 mg/liters its concentration in the atmosphere of a hermetically sealed chamber with a volume of 24 m^3 and 3 men inside reaches the extreme allowable value, based on the 8-hour effect (0.02 mg/liters, on the 9th or 10th day of the experiment (0.023-0.027 mg/liter; Bogatkov, Nefedov, and Poletayev, 1961).

In the studies of G. M. Gorban' et al. (1964) it was shown that the total excretions of products of human vital activity in the atmosphere of a hermetically sealed chamber, converted to a 24-hour value, are as follows: ammonia, 297.6 ± 155.6 mg; carbon monoxide, 278.07 ± 160.8 mg in non-smokers and 417.04 ± 211.5 mg in smokers; hydrocarbons (total), 504.66 ± 333.2 mg; aldehydes, 0.59 ± 0.28 mg; ketones, 232.2 ± 132.8 mg; mercaptans and hydrogen sulfide, 4.95 ± 1.11 mg; fatty acids, 89.45 ± 11.5 mg. The contents of some of these substances in the air of the chamber, in particular CO, ammonia, hydrocarbons and aldehydes, by the 24th hour of the experiment may exceed the maximum allowable concentrations set for the air in industrial establishments.

In these same studies, L. T. Poddubnaya determined the content of several gaseous toxic substances in the atmosphere of a hermetically sealed

chamber with a volume of 5 m^3 in which a human being had stayed for 5 hours. The tests employed practically healthy men aged 20-23. From the moment of sealing the chamber, the oxygen content was kept at the 20-21% level by adding it constantly from a tank through a reducing valve and rheometer. The amount of carbon dioxide in the chamber rose constantly as a measure of its production by the subjects so that by the 5th hour of the experiment it had reached 2-2.5% (Table 27).

TABLE 27. CONTENT OF CERTAIN TOXIC SUBSTANCES IN THE ATMOSPHERE OF A HERMETICALLY SEALED CHAMBER OCCUPIED BY A HUMAN BEING FOR 5 HOURS.

Substances	Number of determination	Concentration of the substance in the air mg/liter ± T	Value of the maximum allowable concentration (State Standard 245-63, mg/liter)
Amm and its compounds	25	0,0026 ± 0,0014	0,02
Aldehydes.	22	Traces	
Ketones.	25	0,023 ±0,007	0,2
Hydrogen sulfide and mercaptans.	25	Traces	—
Organic acids converted to acetic acid.	24	0,0012 ±0,0005	0,005
Carbon monoxide in experiments with non-smokers	12	0,012 ±0,0007	0,02
In experiments with smokers	12	0,018± 0,01	0,02
Total hydrocarbons converted to C.	20	0,026 ±0,013	0,3

Commas indicate decimal points.

It is clear from the latter Table that a 5-hour sojurn by an individual in a hermetically sealed chamber is accompanied by the accumulation in the atmosphere of ammonia and its compounds, aldehydes, ketones, hydrogen sulfide and mercaptans, organic acids, carbon monoxide and hydrocarbons. After 5 hours of the experiment, the concentrations of organic acids, ketones, CO and hydrocarbons in the air of the chamber nearly reaches the limit of the maximum level permissible for an 8-hour isolated effect of each of these substances. The combined effect of the substances listed in Table 27 caused a state of somnolence in the majority of subjects; some of them complained of headaches and feelings of exhaustion. /87

All of this indicates that the concentrations given for the products of vital activity, even in short-term exposures, are not harmless to the human organism. This view is supported by the experiments of O. A. Naumova (1960) which showed that products of human vital activity accumulated in the air of a closed environment (total oxidizability of the air = 10-15 mg of O_2/m^3), even for a 2-hour exposure of animals, produces definite disturbances of higher nervous activity in the latter.

CHAPTER VI

EFFECTS OF THE PRODUCTS OF THE VITAL ACTIVITY OF AN ORGANISM ON EXPERIMENTAL ANIMALS

A Brief Discussion of the Toxicological Characteristics of the Principal Gaseous Products of Vital Activity

As shown in the preceding chapters. the primary gaseous products of /88
vital activity, given off by man, and the secondary products from decomposing urine, feces, and sweat contain a very complex mixture of different chemical substances having a variety of effects on the organism. Their composition includes both inorganic compounds (CO, CO_2, ammonia, hydrogen sulfide) and organic ones (acetone, phenol, indole, aliphatic amines, hydrocarbons, alcohols, organic acids, etc.). In order to demonstrate the possible nature of the toxic effect of this complex combination of substances on the organism, we must first examine some brief data on the toxicology of these compounds.

Acetone. The toxic effect of acetone is characterized by a preferential irritation of the central nervous system, disturbances of conditioned reflex activity and the development of ketosis. In the event of chronic intoxication, there is a decrease in body weight, anemia, disturbance of liver functions, ketonemia and ketonuria (Patti, 1963). Irritation of the upper respiratory passages, conjunctivitis, headache and dizziness are also observed. Hematological studies reveal leucocytosis followed by leucopenia (Okuneva, 1930). The albumin level in the blood serum decreases (Bacin et al., 1960). The marked effect of acetone causes increased activity of blood catalase during the first hours and a decrease in its activity later on (Dienes, Tofalvie and Csotos, 1965) as well as a change in its cholinesterase activity (Mikhaylov, Pilipyuk, 1968).

Data on the toxic concentrations of acetone for various types of laboratory animals are given in Table 28.

TABLE 28. TOXICITY OF ACETONE FOR VARIOUS LABORATORY ANIMALS (LAZAREV, 1963; PATTI, 1963)

Animal	Acetone concentration mg/liter	Exposure in hours	Degree of toxic effect
White mice	150	2,0	Death
	110,0	1,0	»
	48,0	1,5	Narcosis
	40,0	3,0	
	40,0	2,0	Lateral position
White rats	300,0	1,75—2,25	Death
	100,0	4,5—5,5	»
	100,0	1,75—2,0	Absense of corneal reflex
	50,0	2—2,5	Disappearance of reflexes
	25,0	1,5—3,0	Initial signs of poisoning
	10,0	8,0	No visible signs of poisoning
Guinea pigs	95,0	4—8,0	Pronounced intoxication
	47,0	8—9	Disappearance of reflexes
Rabbit	1,25—2,5	2,0	Change in rate of development of reflex muscle stress
Cat	8—10,0	5,0	Irritation of mucous membranes
Dog	5—6	7	Does not produce disturbances of conditioned reflexes

Commas indicate decimal points.

At our request, P. A. Kolesnikov conducted special studies on white mice to determine the thresholds of the acute effect of acetone. The parameters of acute toxicity were determined in advance. Experiments were performed on male white mice weighing 20-25 grams. Each concentration of acetone was studied in 10 animals. The acetone content in the air was monitored analytically. The acetone was determined by the method of F. D. Shikhvarger (1954). The results of the studies were analyzed by the method of test probe analysis. To determine the threshold of the acute effect in white mice with 2-hour poisoning by inhalation, the rectal temperature was measured with the aid of a previously calibrated thermocouple and the defensive conditioned reflexes were tested by the method of I. S. Aleksandrov and M. G. Tsybina (1947) in the modification of V. P. Paribok (1954). In addition, the "working capacity" of the animals was determined by measuring the time the animals could hold onto vertical rods. The results of these studies are given below. /89

Acetone concentration, mg/liter	Degree of toxic effect
72.3	CL_{100}
56.5(50.6 + 61.8)	CL_{50}
2.46	Disturbance of "working capacity"
0.7	Drop in rectal temperature
0.15-1.83	Disturbance of conditioned reflex activity

/90

In man, a 5-minute exposure to acetone at a concentration of 22 mg/liter (9,300ppm) causes irritation of the upper respiratory passages (self-observation by Kagan) (Kagan, 1924). A concentration which is almost 20 times less (500 ppm, for 1 minute) also causes irritation of the mucous membranes of the eyes and the upper respiratory passages in man (Carpenter and Smyth, 1946; Nelson et al., 1953; Gomer, 1960).

At a concentration of 0.01 mg/liter for 7-8 hours exposure, acetone produces an increase in the level of ketonic bodies in the blood in man (Kustov, 1967).

Ammonia is an irritant poison. High concentrations of ammonia cause irratation of the central nervous system and can lead to collapse and spasms. Breathing of moderate amounts of ammonia produces bronchitis, tracheitis and laryngitis (Lockens, 1961). Intoxication is accompanied by an increase in the blood ammonia content and leads to irritation of the liver. Death in the case of severe poisoning by ammonia may be the result of the development of cardiac weakness, and is more often the consequence of edema of the larynx or lungs (Lazarev, 1963). Chronic intoxication by ammonia takes the form of catarrh of the upper respiratory passages, accompanied by conjunctivitis and anemia (Letavet, 1964).

Biochemical studies have shown that poisoning by ammonia increases the content of residual blood nitrogen (Recine, 1956), increases the activity of glutamic oxalo-acetic transaminase (L-aspartate: L-oxyglutarate-amino-trans--ferase 2.6.1.1.) and glutamic pyroracemic transaminase (L-ananine: L-oxyglutarate-amino-transferase 2.6.1.2.) and produces toxic hepatitis (Pilgerstorfer, 1963). Increased ammonia content in the blood and tissues causes inhibition of tissue respiration and disturbance of acetylcholine metabolism (Kozlov,

1962). Excess ammonia in the blood leads to increased amination of α-keto-glutaric acid to glutamic, which disturbs the normal function of the Krebs cycle. This in turn leads to accumulation of acetyl coenzyme A and provokes the development of acetonemia and acetonuria (Leytes and Lapteva, 1967). The toxic concentrations of ammonia for laboratory animals are shown in Table 29.

Data on the toxic effect of ammonia on the human organism are shown in Table 30.

TABLE 29. TOXICITY OF AMMONIA FOR VARIOUS LABORATORY ANIMALS

Animals	Ammonia concentration, mg/L	Exposure time in hours	Degree of toxic effect	Source
White mice	3,8 (3,3± ±4,3	2,0	CL_{50}	Alpatov, 1963
	0,05	2,0	Change in oxygen consumption	" "
	0,03	2,0	Change in threshold of nervous and muscular excitation	" "
White rats	7,6	2,0	CL_{50}	" "
	0,085	2,0	Change in threshold of nervous and muscular excitation	" "
	0,025	2,0	Irritation of upper respiratory pathways	" "
Guinea pigs, rabbits, etc.	14,0	—	Rapid death	Lazarev, (ed.), 1963
	7,0	3,0	Death	" "
	3,5—5,0	1,5—4,0	Lung infection	" "
	0,35	4,0	Lung irritation	" "

Commas indicate decimal points.

As Tables 29 and 30 indicate, the human organism is much more sensitive /91
than the animal organism to the toxic effect of ammonia, and reacts to very small concentrations of it.

In addition to its inhalation effects, ammonia has a pronounced local effect on the skin, mucous membranes of the eyes and upper respiratory passages.

Action of ammonia on the skin is characterized by edema, serous vesicles and disturbance of the intactness of the cutaneous covering (Vol'fovskaya and Davydova, 1945).

TABLE 30. EFFECT OF AMMONIA VAPOR ON THE HUMAN ORGANISM

Ammonia concentration, mg/liter	Degree of toxic effect	Source
0,35—07	May be dangerous for life	Lazarev. 1963
0,49	Irritation of eyes	» »
0,28	Irritation of larynx	» »
0,1	Irritation	» »
0,07	Complete suppression of rhythm in EEG	Alpatov, 1963
0,04—0,05	Slowing of respiration, reduction of amplitude of rhythm in EEG	» »
0,02	Change in electrical potential of skin	» »
0,01	No effect	» »
0,0005	Threshold of olfactory preception	Sayfutdinov, 1967
0,00045	Reduction of sensitivity of eyes to light	» »
0,00035	Change in the electrical activity of the brain	» »
0.013 during 7-8 hours	Increased urea content in the blood and increased ammonia content in the urine	Kustov, 1967

Commas indicate decimal points.

The long-term continuous inhalation effect of ammonia has been studied in experiments on white rats. It was found that a 60-day exposure to the effects of ammonia in a concentration of 0.036 mg/liter produces an increase in the content of leucocytes in the blood and a change in oxygen consumption (Alpatov, 1962). In smaller concentrations (0.020 mg/liter) but with exposure for 84 days, ammonia produced a shortening of the time of reflex reactions in white rats, inhibition of the cholinesterase activity and increased excretion of coproporphyrin and ammonia with the urine (Sayfutdinov, 1967).

Aliphatic amines. Of the gaseous product of vital activity of man, the aliphatic amines that are most often found are methylamine, dimethylamine /92 and trimethylamine (Burnazyan. Nefedov, Parin et al., 1967).

The toxic effect of aliphatic amines is characterized by the disturbance of the function of the central nervous system, signs of its excitation with subsequent inhibition, and by spasms. Irritation of the upper respiratory passage is usually noted (Brieger and Hodes, 1951). The aliphatic amines can be arranged as follows according to their degree of toxicity: methylamine, ethylamine, amylamine, dimethylamine, trimethylamine, and triethylamine

(Brunton and Cach, 1885). The toxic concentrations of several aliphatic amines are listed below (Table 31).

TABLE 31. TOXICITY OF SEVERAL ALIPHATIC AMINES FOR LABORATORY ANIMALS

Substance	Animal	Concentration of mg/liter	Exposure in hours	Degree of toxic effect	Source
Methylamine	White mice	2,3	2,0	Death	Lazarev, 1963.
			0,7	Change in characteristic of unconditioned reflex	"
	Rabbit	0,3	0,7	Disturbance of conditioned reflexes	Gorbachev, 1957
	»	0,05	Several minutes	Irritation of upper respiratory passages	"
	Cat	0,2	2,0	Death	Kremneva and Sanina, 1961.
Dimethylamine	White mice	2,3—2,5	2,0	Average lethal concentration	"
		0,07	2,0	Minimum lethal concentration	"
		0,03	2,0	Threshold concentration on basis of duration of swimming	"
		0,005	2,0	50% mortality	"
»	White rats	2,3—2,5	2,0	Disturbances of conditioned reflex activity	"
		0,005	2,0	No disturbance of conditioned reflexes	"
		0,003	2,0		
Dimethylamine	Rabbits	0,40	40 Min	Change in the nature of the flexile reflex	Lazarev, 1963
	Cats	0,4	4 Min		
Trimethylamine	White mice	24,8	2,0	Excessive salivation	Mezentseva, 1956
		19(17.9 - - 22.2)	2,0	84% mortality	Rotenberg and Mashbits, 1967
		14,3	2,0	50% mortality	"
		0,075	5 hours daily for 7 months	16% mortality	"
	White rats			Lymphopenia, enlargement of adrenals, suppression of neurosecretion in the bodies of nerve cells in the anterior hypothala	"

Commas indicate decimal points.

A human being can detect the odor of dimenthylamine at a concentration of 0.0005-0.001 mg/liter, and the odor of dimethylamine [Sic....Tr.Note] at a concentration of 0.0025 mg/liter. The threshold of detection of the odor of trimethylamine is 0.002 mg/liter (Rotenberg and Mashbits, 1967). The thresholds of the irritant effect of mono- and dimethylamine for man, are 0.01 and 0.05 mg/liter respectively, (Lazarev, 1963). Similar results for the toxicity of aliphatic amines are given in the works (William and Sutton, 1963).

Carbon monoxide is a typical anoxic poison which blocks oxygen transport by blood hemoglobin and inhibits tissue respiration. Intoxication by carbon monoxide is characterized primarily by irritation of the central nervous system. Clinical cases of poisoning by carbon monoxide show the existence of an exclusive polymorphism, related to the disturbance of the activity /93
of almost all systems of the organism. In addition to the nervous system, there is also irritation of the cardiovascular system, the respiratory system, the endocrine system, and the hemopoietic system. Changes are described involving the organs of vision and hearing, with irritation of the cutaneous coverings. Biochemical studies reveal disturbances of carbohydrate metabolism (hyperglycemia, etc.), nitrogen metabolism (azotemia, increased ammoniacal coefficient, etc.), water-salt metabolism (hypercalemia, etc.) mineral metabolism and oxygen-alkaline equilibrium, as well as inhibition of a number of enzyme systems. Detailed data on the toxicology of carbon monoxide are to be found in papers by L. A. Tiunov (1953, 1963) and L. A. Tiunov and V. V. /94
Kustov (1969).

Data on the toxic concentrations of carbon monoxide for various types of laboratory animals are listed in Table 32.

The first signs of intoxication by carbon monoxide are noticed 2-3 hours after its effect in a concentration of 0.22 mg/liter in the event of exposure of man to CO in a concentration of 0.88 mg/liter after 45 minutes headache, muscular weakness and nausea are observed. At the same concentration for 2 hours, loss of consciousness and collapse ensue. The action of carbon monoxide /95
for 1 - 1.5 hours in a concentration of 1.8-2.3 mg/liter causes loss of consciousness in man; death is possible (Lazarev, 1963). Thus, as far as the effects of CO are concerned, man is more sensitive to its toxic effects than are laboratory animals.

In long-term exposure to carbon monoxide, according to the data of I. D. Gadaskina et al. (1958, 1961), in concentration of 0.03 mg/liter (100 days, 6 hrs. daily), guinea pigs, rabbits, white rats and mice showed a decline in "working capacity," a change in oxygen consumption, an increase in the number of sub-threshold impulses producing the flexor reflex, unpleasant changes

with respect to the cardiovascular system (rabbits), increased activity of the thyroid gland and lengthening of the time for establishing the breathing rate in measured work (guinea pigs).

TABLE 32. TOXICITY OF CARBON MONOXIDE FOR LABORATORY ANIMALS

Animals	Concentration of mg/liter	Time, hrs.	Effect	Source
White mice	10,9	1,5	CL_{100}	Our data
	8,0	1,5	CL_{50}	» »
	6,0	2,0	CL_{100}	Belyayev (cited in Tiunov
	3,6 (3,2±4,0)	2,0	CL_{50}	and Kustov, 1969)
	2,5	2,0	CL_{16}	» »
	2,0	15 min	Changed conditioned reflex activity	» »
	6,0	3,0	CL_{20-40}	Kagan, 1949
	4,5	3,0	CL_{22}	» »
White rats	18,0	15 min	CL_{100}	Our data
	9,0	15 min	CL_0	»
	4,1	50 min	CL_{100}	Frolov, 1944
	3,0	15 min	Changed conditioned reflex activity	Our data
Guinea pigs	11,5	50 min	Death	Likhachev, 1931
	4,6	135 min	»	
Rabbits	18,0	1,5	Death	Our data
	20,0	1,0	»	
	0,1--0,2	2,0	Increased time for flexile reflex	Zakusov, 1937
Cats	5--6	0,5	Death	Wirth, 1930
	4,6—5,8	45--90 min		Yavich, 1928
Dogs	5,7—11,4	20--30 min	Death	Flyuri and Tsernik, 1931
	0,05 --0,9	1,0	Changed condition reflex activity	Gorsheleva, 1944

Commas indicate decimal points.

Our experiments with male white rats showed that continuous 24-hour poisoning with carbon monoxide in a concentration of 0.012 ± 0.005 mg/liter for 85 days produces inhibition of the growth of experimental animals, erythrocytosis, increased "altitude" ceiling and a change in trainability for physical stress.

In man, inhalation of carbon monoxide in a concentration of 0.11 mg/liter causes many disturbances in the mental sphere (Schulte, 1963). According to the data of V. V. Justova (1967), exposure to carbon monoxide for 7 hours at a concentration of 0.013 mg/liter produces a drop in oxygen comsumption in man, a drop in the coefficient of its usage, an increased number of errors in performing mental tests and changes in the EKG.

Phenol has an unpleasant odor and an irritating and generally toxic effect. It is a protoplasmic poison that affects primarily the nervous system. Its action is accompanied by the development of vegetative reactions, slowing of respiration, nausea, and vomiting. The toxicity of phenol for different animals varies (Table 33). Poisoning of human beings by phenol vapor has been observed when its content in the air was 0.0088-0.0122 mg/liter (Petrov, 1960). Chronic intoxication by phenol is accompanied by irritation of the upper respiratory passages, dyspeptic phenomena, irritability, insomnia, tendency to perspire and kidney damage (Dolgov, 1933). Conjunctivitis, catarrh of the upper respiratory passages, increased fatigability and dizziness have all been observed.

TABLE 33. TOXICITY OF PHENOL FOR LABORATORY ANIMALS

Animal	Concentration mg/l	Exposure hours	Degree of toxicity	Author
White mice	0,02	90 days	CL_0	Thomas and Beck, 1967
	3,7	—	Death	
	3,2	—	CL_0	Lazarev, 1963
White rats	0,02	90 days	CL_0	Thomas and Beck, 1967
Guinea pigs	0,1—0,2	7 hours daily for 20 days	Decrease in weight	Lazarev, 1963
	0,05—0,157	6 hours daily for 71 days	Irritation of lungs, liver and kidneys	Kurnatowski, 1961
White rats	0,005—0,01	60 days continuously	Change in blood cholinesterase activity, disruption of porphyrin metabolism, change in motor chronaxy.	Mukhitov, 1963

Commas indicate decimal points.

Biological studies have shown that phenol causes disturbances of acetylcholine metabolism; it changes the activity of the blood cholinesterase and disturbs prophyrin exchange (Mukhitov, 1963). Phenol also has a local effect that causes dermatitis. The threshold of perception of the odor of phenol is 0.029 mg/m^3. The effect of phenol at a concentration of 0.022 mg/m^3 causes /96
increased sensitivity of the eyes to light in man(Mukhitov, 1963).

Indole has a sharp unpleasant odor. Its toxic effect is characterized by irritating and reflex effects. In 90-day continuous exposure of experimental animals, idole in a concentration of 0.5 mg/liter caused the death of two monkeys out of 16, 5 rats out of 50 and 22 mice out of 100 (Thomas and Beck, 1967). Myeloid leucosis develops in white mice under the influence of indole. In man, idole produces nausea and other unpleasant symptoms (Lazarev, 1963).

Hydrogen sulfide is an anoxic poison that produces tissue anoxia due to blockage of the iron-containing enzyme system. It has a pronounced irritating effect. The toxicity of H_2S differs for various animals (Table 34).

In man, poisoning occurs immediately upon exposure to 1 mg/liter of hydrogen sulfide and death results from prolonged breathing. A 4-hour exposure to hydrogen sulfide in a concentration of 0.006 mg/liter in man produces headache and lacrimation (Arutyunov, 1934). Biochemical studies of poisoning by hydrogen sulfide have involved research on the activity of acid and alkaline phosphatase of desoxyribonuclease II in the nervous system of the rabbit (Kaminski and Mikolojczyk, 1967), and the mineral content of the plasma and tissues (Kosmider and Rogala, 1967).

After chronic exposure to hydrogen sulfide, victims develop bronchitis, /97
conjunctivitis, and disturbances of the function of the gastrointestinal tract; they suffer from disturbances of sleep and visual disorders (Khagen, 1961). There is a possibility of a drop in hemoglobin and erythrocyte levels (Letavet, 1964). Also evident are vaso-vegetative disturbances, increase of reflexes, increased dermographism, arcocyanosis, and tremor of the fingers (Grigor'yev, 1967).

TABLE 34. TOXICITY OF HYDROGEN SULFIDE FOR LABORATORY ANIMALS

Animal	Concentration mg/l	Exposure in hours	Degree of toxicity	Source
White mice	1,15	2,0	CL_{10}	After Lazarev (ed.), 1963
	1,0	5,0	CL_{100}	Ditto
	0,9	5,0	CL_{66}	"
	0,8	5,0	CL_{30}	"
	0,7	5,0	CL_{7}	"
	0,03	90 days	CL_{26}	Thomas and Beck, 1967
White rats	0,03	90 days	CL_{24}	Ditto
	0,02	—	Change in conditioned reflex activity	Shchur (cited in Lazarev), 1963
Guinea pigs	0,2	—	Initial symptoms of poisoning	Ditto
Rabbits	0,8—1,25	1¼—8	Death	"
	0,06	—	Change in flexive reflex	Filippovich, 1950
	0,014	3 weeks, 8 hrs. daily	Irritation, conjunctivitis, corneal defects, changes in gastrointestinal tract.	Weise, 1933
Cats	0,19	-	Irritation of mucous membranes, salivation	Lazarev, 1963
Dogs	0,14—0,21	—	First symptoms of intoxication	Ditto
Monkeys	0,03	90 days	CL_{0}	Thomas and Beck, 1967.

Commas indicate decimal points.

The threshold of olfactory perception of hydrogen sulfide, according to B. K. Baykov (1964), is 0.014-0.03 mg/^{3}. Duan' Fyn'-Zhuy (1959) obtained similar data, 0.01-0.03 mg/m^3. The sensitivity of the eyes to light changes under the influence of hydrogen sulfide in a concentration of 0.012 mg/m^3 (Baykov, 1964).

The Combined Effect of Gaseous Products of Vital Activity

Consideration of the toxic properties of some of the compounds that /98
enter into the composition of the gaseous products of vital activity has shown that they have diverse effects on the organism. It is therefore practically impossible to get an idea of the irritation produced by a mixture of all these substances. We can expect that the mixture will have an unpleasant odor and have an irritating effect. However, the general toxic action of this

mixture will evidently be more complex. We must anticipate that under the conditions of hermetically sealed cabins the chemical substances will act on man for long periods of time. This means that completely new and unexpected effects not manifested by the individual components of the gas mixture may appear. In particular, G. Stokinger (1967) points out that under analogous conditions the narcotic and irritating effects may not appear but there will be disturbances connected with the inhibition of enzyme activity. It is therefore necessary to carry out special studies to investigate the toxicity of the substances under conditions of their prolonged and continuous action (Tiunov and Savateyev, 1962). These studies constitute one of the problems of the toxicology of closed ecological systems (Lazarev, 1967; Kustov and Tiunov, 1968). Another difficulty in the evaluation of the biological effect of a mixture of gaseous products of vital activity is the insufficiency of knowledge of their combined effect. At the same time, the toxic effects of these compounds can either be summed (additive effect), attenuated (antagonism), or intensified. It is also possible for "independent" effects to occur. Besides, these products can interact with one another and with the components of the air medium, with formation of still unknown and unstudied substances.

Carbon monoxide and carbon dioxide. Data on the combined effect of carbon monoxide and carbon dioxide are highly contradictory. Deckert (1929) described an increase in the toxicity of CO in the presence of increased concentrations of CO_2. This was supported in a work by Boltz and Machate (1963). According to the data of Raymond and Marton (1954), the toxic effect of carbon monoxide (0.04%) increases by a factor of 2 in the presence of 1% carbon dioxide. However, F. A. Ivanova and F. M. Chebotarev (1963) were unable to reproduce this finding. Evidently the increase in the toxic effect of carbon monoxide in the presence of carbon dioxide depends on concrete combinations of these poisons. The high concentrations of CO_2 which cause hyperoxia, acidosis and hyperventilation of the lungs will promote the appearance of the toxic effects of carbon monoxide.

Ammonia and carbon monoxide. The combined effect of ammonia and carbon monoxide has been studied in experiments on white mice (Kustov and Mikylov, /99

1963). Intensification of the toxic effects was observed in acute experiments. The intensification effect was preserved in the case where the mortality of the animals served as the criterion, and also when the activity of the blood cholinesterase of the experimental mice served as the criterion of the action of the poison. The toxic effect of carbon monoxide in the presence of ammonia vapor was intensified about 1.6 times. In observations made on human subjects, however, the combined effect of carbon monoxide and ammonia was "independent."

Ethanol and carbon monoxide. The combined effects of ethanol and carbon monoxide are summed (Pecora, 1959). Administration of ethyl alcohol to dogs for a period of 21 weeks led to an increase of its content in the blood by 0.15%; it also intensified the chronic effect of CO, causing a more pronounced drop in the lipoproteid level. An increased toxic effect of CO in combination with ethanol was also described by Wittgens (1958) and Mallash and Roeseler (1961).

Carbon monoxide and hydrogen sulfide. Hofer (1926) described the increase in the toxic effects of CO and hydrogen sulfide in their combined action in acute experiments on white rats. This intensification of the toxic effects had the nature of a summation (Lazarev, 1938). However, this question requires further experimentation on the level of the threshold concentrations in chronic experiments.

Carbon monoxide and methane. In the combined effect of CO and methane, L. I. Morozova (1965) noted a smaller degree of manifestation of morphological changes in the organs of white mice than in the case of separate action of the poisons. A weakening of the toxic effect of carbon monoxide in combination with methane was also observed by A. G. Prokhorenko (1965), using biochemical indicators as criteria. It was found that under the combined influence of carbon monoxide and methane the level of ammonia and the content of nitrogen amide and glutamine in the brain changed to a lesser degree than with the separate action of these poisons (Prokhorenko, 1968).

Hydrogen sulfide and methyl mercaptan. The combined effect of hydrogen sulfide and methyl mercaptan was studied in chronic experiments with mice

at concentration of 0.03 and 0.1 mg/liter, respectively. It was found that the combined effect of these poisons is synergistic and characterized by summation of the toxic effects (Thomas and Beck, 1967).

Indole, hydrogen sulfide, methyl mercaptan, skatole. In the above, we have described the combined action only of individual pairs of gaseous products that enter into the composition of the complex of human excreta. Therefore, it was extremely interesting to read the work of A. Thomas and K. Beck (1967), who /100 performed a study on various forms of laboratory animals to determine the toxicity of a mixture of four substances in a 90-day continuous exposure. The following were the concentrations of the poisons in the atmosphere: indole, 0.5 mg/liter; hydrogen sulfide, 0.03 mg/liter; methyl mercaptan, 0.1 mg/liter and skatole, 0.01 mg/liter. Taken separately, the same substances in the same concentrations, in a 90-day exposure experiment with monkeys, produced the following effect: indole, death of 2 monkeys out of 10; hydrogen sulfide: no deaths; methyl mercaptan: death of 4 monkeys out of 10. In combined action, these poisons caused deaths in 16 out of 20 monkeys. Similar data were obtained in experiments on rats and mice. Indole caused the death of 5% of the rats and 22% of the mice, hydrogen sulfide killed 12% of the rats and 26% of the mice, and the figures for methyl mercaptan were 10 and 43%, respectively. In the combined action of these poisons, the mortality of the rats was 64% and that of the mice, 99%. On the basis of these tests we can conclude that the toxic effects of indole, hydrogen sulfide and methyl mercaptan are summed.

Hence, an analysis of the data on the combined effect of individual combinations of the chemical substances that enter into the composition of the mixture of gaseous products of vital activity shows the presence of very complex relationships. In fact, in this complex mixture of poisons there is also a summation of the toxic effects, an independent effect, and a weakening of the effect. All of this still does not allow us to calculate the total toxic effect of a combination of these poisons. To do this, we would have to conduct studies to investigate the combined effect of many dozens of combinations of chemical substances. At the same time, the existence of the diverse character of the combined effect, observed when considering only a small part

of the possible combinations, does not allow us to assume any one form of combined effect for the entire complex, for example, summation or "independent" effects. It therefore appears worthwhile to conduct special experiments on laboratory animals to investigate the nature of the biological effect of the entire complex of chemical substances that are excreted from the organism in the process of its vital activity.

The Biological Action of the Complex of Gaseous Products of Vital Activity

On our request, T. S. Kolosova conducted special studies on white rats. The choice of these animals was not a chance one. White rats are resistant to infection and are widely used in studies on the physiology and biochemistry of nutrition and in the study of chronic intoxication (Innes, 1965). The reaction of many biochemical systems in the white rat is closer to that of man than in other types of laboratory animals (Linyucheva and Tiunov, 1966).

In the experiment, 20 white rats weighing 100-150 g were used; they /101
were placed in a metal hermetically sealed chamber with a volume of 0.24 m^3. Excess CO_2 was removed by KhPI absorbent. Oxygen was supplied to the chamber automatically. The chamber was not cleaned for the entire duration of the experiment. This produced an increase in the air not only of the primary gaseous products of vital activity, liberated from the organism, but also the secondary ones, given off by the urine and feces. The concentration of toxic substances in the air of the chamber was monitored analytically.

The ammonia content was measured by Nesler's reagent (Zhitkova, 1954); alkyl amines were determined by the method of G. S. Salyamon (1963), acetone was measured by the method of F. D. Shikhvarger (1954), phenol was determined colorimetrically by diazotized paranitroaniline (Yefremova, 1954), carbon monoxide was measured with the aid of indicator tubes on the UG-2 apparatus (Filyanskaya, 1960), while oxygen and carbon monoxide were measured with the aid of the Orsa-Lunge apparatus.

As controls, we used animals kept in a vivarium. The temperature in the chamber was 18-22°C, the relative humidity was 81-85%, the oxygen content was 19% on the average, and the carbon dioxide content was about 1%. Data on the

content of the gaseous products of vital activity in the chamber are listed in Table 35.

TABLE 35. CONCENTRATION (in mg/m^3) IN THE CHAMBER OF GASEOUS PRODUCTS OF VITAL ACTIVITY

Substance	Maximum	Minimum	Substance	Maximum	Minimum
Carbon monoxide	50,0	22,0	Phenol	0,26	0,07
Ammonia	103,0	22,3	Acetone	2,7	0,3

The animals were weighed periodically (once a week). The experiment lasted 26 days. The animals were subjected to detailed examination at the end of the experiment. /102

The results obtained indicate a definite delay and complete inhibition (mainly toward the end of the experiment) of the growth of the experimental animals (Figure 18, Table 36). Thus, a widely employed integral factor studied in toxicology, the dynamics of the change in body weight (Yelizarova, 1962), gives a clear indication of the toxic effect of a mixture of gaseous products of vital activity. On the 26th day, 10 experimental animals were killed by decapitation. A macroscopic investigation revealed no changes in the internal organ[13]. We determined the weight coefficient of the internal organs (i.e., the ratio of the weight of the organs in grams to the body weight in kilograms) (Table 37). In many instances this integral index allows an estimate of the degree of chronic toxicity of a given poison (Rylova, 1964).

[13]The pathological anatomical study was performed by G. A. Lemesh.

TABLE 36. WEIGHT OF EXPERIMENTAL ANIMALS IN THE COURSE OF EXPOSURE TO THE ACTION OF A COMPLEX OF GASEOUS PRODUCTS OF VITAL ACTIVITY

Time, days	Weight, % of original		Time, days	Weight, % of original	
	Experiment	Control		Experiment	Control
Before experiment	100.0	100.0	After 14	105.0	126.3
After 7	104.0	110.5	After 21	107.0	134.2
			After 25	101.4	134.0

TABLE 37. WEIGHT COEFFICIENT OF INTERNAL ORGANS IN WHITE RATS DURING EXPOSURE TO EFFECT OF GASEOUS PRODUCTS OF VITAL ACTIVITY

Organ	Experiment	Control (after Rylova, 1963)	Organ	Experiment	Control (after Rylova, 1963)
Lungs	7.91 ± 0.44	2.8-6.75	Thyroid gland	0.97 ± 0.09	0.15-0.55
Heart	4.94 ± 0.14	2.85-4.4	Adrenal	0.35 ± 0.03	0.21-0.38
Liver	38.37 ± 1.77	28.2-43.5			
Kidneys	9.44 ± 0.28	6.3-8.36			

Of the internal organs, only the thyroid gland had a weight coefficient for the experimental animals that was significantly higher than for the intact ones.

A hematological study was also performed, whose results are shown in /103 Table 38.

As the Table shows, the experimental animals showed a drop in the erythrocyte content in the peripheral blood. This observation agrees with the data of A. Thomas and K. Beck (1967), who observed phenomena of memolysis in experimental animals exposed to the combined effect of gaseous products of vital activity (indole, hydrogen sulfide, methyl mercaptan and skatole).

In addition, we also measured the blood content of reduced glutathione and sulhydryl groups in the animals. The reduced glutathione was measured by the method of Benesch (1950), while the sulhydryl groups were analyzed by the method of amperometric titration according to Koltdoff and Harris (1946). The content of sulhydryl groups in the blood of experimental animals did not differ

from that of the controls. The data on the amount of reduced glutathione in the blood indicates some increase in its content in the experimental animals.

TABLE 38. EFFECT OF GASEOUS PRODUCTS OF VITAL ACTIVITY ON THE HEMATOLOGICAL INDICES IN WHITE RATS

Experiment								Control							
Erythrocytes, millions	Leucocytes, thousands	Lymphocytes	With segmented nuclei	Bacilliform nuclei	Basophilic	Monocytes	Eosinophils	Erythrocytes millions	Leucocytes, thousands	Lymphocytes	With segmented nuclei	Bacilliform nuclei	Basophilic	Monocytes	Eosinophils
4,2	2,65	62,0	35,0	1	—	1	1	5,6	5,35	83	15	1	—	1	—
3,8	2,4	86	12	1	—	1	—	4,1	4,25	83	15	1	—	1	—
3,6	5,65	69	26	2	1	2	—	5,4	3,5	80	16	2	—	1	1
4,9	2,9	71	27	1	—	1	—	5,6	4,5	77	20	2	—	2	—
4,1	2,4	74	23	2	—	—	1	5,7	4,25	76	20	1	—	2	1
2,4	7,4	70	27	—	1	1	1	3,5	5,0	72	14	2	—	2	—
4,9	6,2	73	24	—	1	1	1	5,9	6,1	80	18	—	—	1	1
3,8	3,5	72	24	1	—	1	—	6,7	5,75	79	16	1	—	2	1
5,5	6,0	Not determined						7,2	8,7	Not determined					
3,2	7,0	»						6,1	3,8	»					
M4,0	4,61							M5,58	5,1						

Commas indicate decimal points.

A study of the activity of blood catalase was conducted using the method of A. N. Bakh and R. S. Zubkova. It was found that the catalase index of the blood in experimental animals was 1.1, while in the controls it was 0.74, i.e., there was an increase in blood catalase activity. Finally, we studied the oxygen consumption of the experimental animals. The determination of oxygen /104 consumption was conducted according to the method of S. V. Miropol'skiy. It was found that in the experimental animals, the oxygen consumption per 100 g of weight was 9.82 ± 0.21, while in the controls it was 5.14 ± 0.19 ml, i.e., there was a significant increase in oxygen consumption, which may be the consequence of compensatory reactions in conjunction with the development of some anemia. The increase in the size of the thyroid gland in the experimental animals also agrees with this.

Special studies were conducted to determine how these indices change when the period of exposure is increased to 60 days. It was found that the drop in the content of erythrocytes is replaced by erythrocytosis (prior to

the experiment, the erythrocyte content was 6.4 ± 0.41 million; after 60 days, it was 7.1 ± 0.20 million); the activity of blood catalase was sharply reduced (0.77 ± 0.003 before the experiment, 0.59 ± 0.004 after 60 days). Oxygen consumption (O_2 per 100 g of wt.) remained high (5.6 ± 0.4 prior to the experiment, 7.2 ± 1.2 ml afterward).

Thus, on the basis of our experiments and the data in the literature, we can attempt to list a number of characteristic signs of the biological activity of the complex of gaseous products of vital activity. This complex of foul-smelling, irritating and anoxic poisons causes disruption of higher nervous activity and interruption of conditioned reflexes (Naumova, 1960). It disrupts acetylcholine metabolism. The generally toxic effect of this complex is manifested in the inhibition of the growth of experimental animals. Anemia and erythrocytosis appear, and there is increased oxygen consumption. Catalase activity undergoes cyclic changes. These changes in catalase activity may be related to the development of hemolysis. The composition of the complex of products of vital activity includes at least three compounds that inhibit the activity of catalase: hydrogen sulfide (Nicholis, 1961), acetone (Dienes et al., 1965) and ammonia (Kustov and Mikhaylov, 1963). A drop in catalase activity leads to the appearance of effects of endogenic hydrogen peroxide that promote the decomposition of hemoglobin, the formation of Heinz bodies (Brenner and Allison, 1955)[14] and subsequent hemolysis. The drop in the erythrocyte content obviously leads to the development of compensatory reactions: a rise in oxygen consumption, an adaptive increase of catalase activity and the development of erythrocytosis. Prolonged exposure leads to more marked changes and disruption of the compensatory mechanisms. Thomas and Beck (1967) considered the biological effect of a complex of gaseous products of vital activity as a sign of non-specific stress effects. The study of the biological effect and the complex of gaseous products of vital activity is a very complicated problem deserving of further study. In the present chapter, we have merely attempted to draw inferences from the material presently available.

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[14] The formation of Heinz bodies under the influence of products of vital activity was described by Thomas and Beck (1967).

CHAPTER VII

PRINCIPLES FOR ESTABLISHING THE PERMISSIBLE LIMITS OF CONCENTRATION OF THE PRODUCTS OF VITAL ACTIVITY FOR THE ATMOSPHERES OF HERMETICALLY SEALED CHAMBERS. THRESHOLDS OF TOXIC EFFECT OF PRODUCTS OF VITAL ACTIVITY ON THE HUMAN ORGANISM.

The problem of establishing the permissible limits of concentration /105
(PLC) of harmful impurities is of extreme importance for the atmospheres of sealed chambers. Within the boundaries of this considerable and difficult problem, the basic aspects of which we considered in a discussion of certain problems of space toxicology (Kustov and Tiunov, 1968) an important place must be given to the experimental determination of the PLC of the toxic substances which constitute the final or intermediate products of human vital activity.

The first hygienic standard limiting the content of products of human vital activity in the air of living quarters was established in 1858. By a purely empirical path, Pettenkofer came to the conclusion that carbon dioxide content can serve as an indirect indicator of pollution of the air by organic products. The upper limit of the CO_2 content in the air of living quarters due to the presence of persons therein must not exceed 1% (Pettenkofer, 1858).

This hygienic standard has retained its validity until the present day.[15] It is used for calculating the required change of air in buildings and serves as a criterion for estimating the purity of room air and the operation of ventilation systems.

However, the content of carbon dioxide in the air of living quarters is not the sole criterion of contamination of the latter.

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[15] O. V. Yeliseyeva (1966), on the basis of a study of the biological effect of small concentrations of CO_2, recommends that the maximum single concentration for the air in living quarters and occupied buildings be 0.1% CO_2, and the average daily amount should be no more than 0.05%, regardless of the source.

F. F. Erisman (1887) emphasized that the odor of the air in a room can serve as a criterion of its purity. "Room air," wrote Erisman, "that has some kind of noticeable odor must be pronounced not clean and unsuitable for breathing." Therefore, "the sense of smell is a good guide in evaluating /106 the soundness of the air in living quarters."

Another criterion of the pollution of room air by the products of vital activity, in Erisman's opinion, might be the quantity of a certain substance excreted by an individual or his clothing; for example, water vapor, organic substances, ammonium compounds and so on, but under the inapplicable condition that "the amounts of these substances in the air increase in proportion to the number of persons located in a given room and in proportion to the actual spoilage of the air." Erisman felt that the content of organic substances in the air of living quarters was the best criterion for its pollution, since "on it obviously depends the specific odor and the general characteristic properties of this air." However, inadequate investigation of the chemical nature of these compounds and the lack in those days of a reliable method of determing them (organic substances are determined according to the total oxidizability of the air) did not allow this criterion to be used to evaluate the pollution of the air of living quarters by the products of human vital activity (Erisman, 1887; Levashov, 1895).

M. A. Khomutova (1952), who studied the accumulation of harmful chemical impurities in the air of living quarters, came to the conclusion that ammonia can serve as a direct indicator of the pollution of air by products of human vital activity. The basis for such a conclusion was, firstly, the fact that the ammonia content in the air of a room has been reliably correlated with the number and length of stay of persons in it. Secondly, a comparison of the concentrations of ammonia with the odor of the air and the content of CO_2 in it has shown that the air of living quarters "gives an impression of staleness" and has a weak specific odor when the ammonia content in it reaches 0.01 mg/liter. The CO_2 level is then below its permissible level for the air in living quarters (0.1%).

The above mentioned integral indices of the pollution of the air in living quarters by products of human vital activity as well as the methods of establishing them cannot be applied in evaluating the permissible content and the hygienic standard for toxic substances in the artificial atmosphere of a hermetically sealed system.

The prolonged and continuous stay of a human being under these conditions has required the devising of a fundamentally new approach and new criteria for standards of optimum conditions of habitation. As far as the values of the PLC of toxic substances are concerned, including the products of vital activity, we must consider that these values must protect not only against the development of severe or chronic intoxication but also must ensure a level of working capacity of man such as is required for carrying out required activity (Genin and Shepelev, 1964; Kustov and Tiunov, 1968; Kitzes, 1959). /107

In the opinion of O. G. Gazenko and A. M. Genin (1967), in the case of a brief stay of an individual in a hermetically sealed system, the hygienic standardization of chemical substances may proceed without determination of the firm boundaries of the allowable concentrations. In case of necessity, these concentrations can even lead to certain functional changes under the condition that after the individual emerges from the hermetically sealed system these changes will not lead to pathological disorders (Genin and Shepelev, 1964). We consider the viewpoints expressed to be all the more justified since, in the opinion of certain investigators (cf. Truhaut, 1965) the accuracy of the methods presently used for establishing the PLC for harmful substances is insufficient and variations of the standard values by factors of 3 or 4 can be disregarded in many instances.

However, as the length of time spent by man in hermetically sealed cabins with artificial atmospheres extends to several months, the problem of high accuracy in the working out of the thresholds of permissible concentrations of toxic substances become more acute. We must concur with the opinion of A. M. Genin and Ye. Ya. Shepelev (1964) that in standardizing the harmful impurities for such conditions one must "proceed on the basis of the

inadmissibility of prolonged functional changes and long-term disturbance of the constancy of the internal environment of the organism."

Thus, the experimental establishment of the PLC for the atmosphere of a closed environment raises a number of problems which must be considered.

The first problem consists in the fact that if, for certain reasons, the PLC of chemical substances devised for industrial enterprises and based on 8-hour exposure for a 40-hour work week cannot be applied to the conditions of continuous contact with these substances for several months (Nefedov and Zaloguyev, 1967; Kustov and Tiunov, 1968; Clemedson, 1959; Schaefer, 1959; Honma, 1961; Hendel, 1964; Stokinger, 1965), what sort of changes and additions must be made in the existing system used in industrial toxicology for determining the PLC to make it usable for the conditions of hermetically sealed environments?

"The provisional methodical instructions for establishing the experimental studies for determining the PLC of harmful substances in the air of industrial establishments" (1965) provide for setting up acute, subacute and chronic experiments on certain kinds of laboratory animals. The orientational PLC established experimentally is refined further by clinical and statistical observations of workers. In the event of an increase in the sick rate and recording of signs of chronic intoxication, the PLC levels are reduced. Thus, for example, the PLC for carbon monoxide for industrial establishments during the period from 1943 to the present time has changed gradually and has dropped from 0.04 to 0.02 mg/liter (Levina, 1948). Such an approach hardly seems applicable in working out the PLC for the atmospheres of hermetically sealed environments. /108

This does not mean, however, that this method of establishing the PLC of toxic substances for air in industry is completely unsuited for establishing the standards for harmful impurities in the atmosphere of hermetically sealed environments. On the contrary, we suggest that this system (after the introduction of certain refinements and changes) could serve as the basis for setting the standards for toxic substances for the atmospheres of hermetically sealed environments.

The PLC value for industrial enterprises depends mainly on the accuracy of determination of the threshold concentration in chronic experiments on animals. Another factor of considerable importance is the value of the coefficient of transition (reserve) from the threshold concentration to the allowable limit. In setting the threshold concentrations, a critical factor is the choice of criteria of the toxic effect (Kurlyandskaya and Sanotskiy, 1965). Integral and specific indices usually are used as such criteria in industrial toxicology.

However, a greater degree of accuracy in determining the threshold concentration under the conditions of prolonged continuous exposure to toxic substances can be achieved by using criteria discussed separately, i.e., enzymatic indices.

According to the data of Stokinger (1965, 1967), prolonged and continuous exposure to poisons in small concentrations is limited mainly to an effect on the enzymatic activity. For a threshold, one does not use a concentration which produces any noticeable changes, but only one which can have significant importance for the organism. These include changes in the activity of the key enzymatic systems, disturbances of the activity of the enzymatic systems which are accompanied by changes in the content of their substrates, or disturbance of such systems which causes a drop in the resistance of the organism to stress effects. We have performed a detailed study of the problem of estimating the significance of enzymatic indices in determining the threshold concentrations of toxic substances, in a special paper (Tiunov and Kustov, 1969).

In industrial toxicology, the value of the coefficient of transition from the threshold concentration to the permissible limit is calculated for each substance separately, on the basis of the degree of its dangerousness. The latter is determined on the basis of an estimate of the zone of severe and chronic effect of the substance with consideration of the possibility of inhalation of the poison (Sanotskiy, 1962).

For the conditions of prolonged and continuous exposure, it is necessary /109 to introduce additional coefficients which quantitatively take into account

the visual differences in the degree of the reactions of the enzymatic systems in man and experimental animals (Tiunov, 1967; Hays, 1965). Similar comparisons of the reactions of experimental animals and man are conducted with the use of different kinds of functional tests to determine the threshold concentrations (MacNamara, 1967).

Once the PLC has been determined in animal experiments to the point that it can be recommended in practice, it must be tested on human volunteers in strictly controlled experiments of suitable length. In many cases, the experiments on volunteers can have independent significance. Thus, the PLC of certain toxic substances of natural human metabolism can be established directly in experiments on human beings (Kustov, 1961, 1962, 1967). The grounds for such an approach to the standardization of the products of human vital activity are the following facts.

All toxic substances excreted by the organism with the expired air, urine, feces, intestinal gases and the substances which enter into the composition of the secretion of the sweat and sebaceous glands of the skin and are also formed by microbial decomposition of organic compounds contained in the above human excreta are not foreign to the human organism. One part of these substances is formed in the process of normal metabolism and is always present in the internal medium of the organism. The other substances are always "around" the human organism at all stages of its evolutionary development.

Therefore, in the process of evolution in the human organism special "functional systems" have arisen (Anokhin, 1962) which steadily maintain the content of toxic endogenous products at a level which is not dangerous to the organism, and "functional systems" which ensure rapid detoxication of toxic products of vital activity that enter the organism from the external environment surrounding it. The reliable functioning of these systems also explains the fact that such highly toxic chemical substances as (for example) carbon monoxide, ammonia, acetone, indole and other components of human metabolic excreta do not produce signs of intoxication at the usual levels of their existence.

However, the physiological possibilities of any "functional system" are not unlimited. In the event of increased formation of a toxic substance in the organism, in the event of prolonged disturbance of its excretion, and also in the event of extreme or prolonged entrance of it into the organism from outside, overstress may occur with exhaustion of the corresponding "functional system" leading to the development of intoxication. Overloading /110 of this system may be revealed by an increased amount of the endogenic product (significantly higher than its physiological level) or of another product of vital activity in the biomedia of the organism, as well as by certain changes in the biochemical or physiological reactions.

These changes in homeostasis, in our opinion, may be viewed as signs of a critical level of some product of vital activity in the medium surrounding an individual. We therefore suggest that we can use as the PLC of a given product of vital activity, that concentration of it in the air whose action for a certain period of time is revealed by a significant accumulation of the substance in the organism and certain changes in the biochemical and physiological indices. The physiological significance of these changes may not be great. Their existence must be taken into account, however, since it indicates the mobilization of the protective-adaptative reactions of the "physiological system" which maintains a stable condition in the organism relative to the action of the toxic substance. Before determining the PLC of the products of vital activity with the participation of human volunteers, we must first determine the following:

- The physiological level of the endogenic substance[16] in the blood, urine, feces and (for substances which are excreted primarily through the lungs) the expired air.

- The "critical" content of this substance, i.e., that concentration of it in the urine, blood and feces at which the protective-adaptative biochemical

[16] In addition to determining the actual endogenic products, it is desirable to determine also the substances accompanying it. For example, in addition to acetone, ketonic bodies should be measured; in addition to NH_3, the content of urea, and so on.

and physiological reactions occur; we must find that concentration at which a critical concentration of the substance in the organism can be reached in a given time.

The level of this concentration, in our opinion, can be recommended as the PLC for that time interval.

The experiments of V. I. Mikhaylov, Z. I. Piliplyuk, V. S. Georgiyevskiy, B. S. Katkovskiy and A. N. Kalinina, using a group of male volunteers 20 to 22 years old, established the physiological levels and the critical contents of acetone and ammonia in the blood and urine.

According to the data of V. I. Mikhaylov and Z. I. Piliplyuk (1968) the physiological level of ketonic bodies in the blood on the average is 6.6 ± ± 2.2 mg% and in the urine, 3.6 ± 1.2 mg%; a 6-7 hour exposure to acetone in a concentration of 1 mg/m^3 did not alter the content of ketonic bodies in the blood and urine of the subjects and did not lead to important changes in the catalase activity of the blood, the activity of the blood cholinesterase, the EKG, the acetone content in the expired air, etc.

Under the influence of a 10 mg/m^3 concentration of acetone, the level /111
of ketonic bodies in the blood at the 6th hour of the experiment reached 15.5 ± 1.42 mg% and was 4.0 ± 1.08 mg% in the urine. At the same time, the acetone content in the expired air increased from 0.0002 to 0.0012 mg/liter. By the 6th hour of the experiment, the subjects showed a statistically significant change in the activity of whole-blood cholinesterase, some changes in the function of the external respiration (increased coefficient of oxygen consumption) and a drop in energy consumption in 1 hour from 93.0 ± 4.7 to 73.3 ± ± 4.4 kcal ($0.05 > p > 0.02$). Since the appearance of these changes may indicate a "switching on" of the protective-adaptative reactions, the experiment was terminated. Sixteen hours after the end of the experiment, the changes observed in the subjects had disappeared.

These experiments showed that the "critical" level of ketonic bodies in healthy young people is 15.5 ± 1.42 mg% on the average. Under the influence of acetone, it can reach a concentration of 10 mg/m^3 in 6 hours. Therefore, the level of the PLC of acetone in the air of a hermetically sealed chamber

to which an individual is to be exposed for 6-7 hours clearly must not exceed 10 mg/m^3.

The experiments of V. I. Mikhaylov, Z. I. Piliplyuk, V. S. Georgiyevskiy and others (1968) have shown that for healthy young men aged 20-22 the normal urea content in the blood is 23.6 ± 1.4 mg% on the average. The ammonia level in the blood can reach 65 ± 8.3 mg%, while the urea in the urine can reach 21.9 ± 1.9 mg/ml. An 8-hour exposure to ammonia in a concentration of 0.003 mg/liter had no discernible effect on these values. Therefore, the slight changes in the function of the external respiration (tendency to reduction of oxygen consumption, a tendency to more rapid breathing) that took place during these experiments were correctly attributed by the authors to the reflex effect of ammonia.

However, after exposure to ammonia for 8 hours in a concentration of 0.013 mg/liter, when the content of urea in the blood reached 39.3 ± 3.8 mg% on the average, and the urea and ammonia levels in the urine were 29.9 ± 3.9 mg/ml and 99.1 ± 8.0 mg% respectively, functional changes in the cardiovascular system (slight bradycardia) and the function of external respiration (decreased consumption of oxygen in comparison with a background experiment by up to 19%, increased rate of breathing) became clearly evident. These changes all vanished some 16 hours after the experiment.

These experiments showed that the urea level in the blood was 39.3 ± 3.8 mg%, while the ammonia and urea levels in the urine were 99.1 ± ± 8.0 mg% and 29.9 ± 3.9 mg/ml, respectively, and were therefore "critical." Levels such as this are reached with an 8-hour exposure to ammonia in a concentration of 0.013 mg/liter, so that the level of the PLC in the air of a heremetically sealed chamber for this period of time clearly must not exceed 0.01 mg/liter. The close agreement of the concentrations given above for acetone and ammonia with values for their PLC obtained through an experiment with animals and conducted under industrial conditions (cf. State Standard No. 245-63) leads us to assume that this method of establishing the PLC of the products of natural human metabolism is correct.

As far as the endogenic substances excreted from the human organism mainly through the lungs are concerned (CO_2, carbon monoxide), the "critical" index for standardization must be their content in the expired air (Kustov, 1961, 1962, 1967; Nefedov and Zaloguyev, 1967; Gorodinskiy et al., 1967; Sedov and Mazin, 1968), since in the event of equality of concentrations of these products in the surrounding medium and expired air, conditions are created for their accumulation in the organism and the appearance of the initial signs of autointoxication.

We tested this view in specially designed experiments conducted jointly with V. I. Mikhaylov, L. T. Poddubnaya, Z. I. Piliplyuk et al. (Kustov et al., 1962, 1965).

In these experiments it was confirmed that carbon monoxide in concentrations equal to its content in the expired air of young non-smokers [0.011 mg/liter (Kustov et al., 1962)] creates certain conditions for its accumulation in the human organism and by the 8th hour of exposure leads to certain changes in biochemical and physiological reactions. In particular, by the 8th hour of the experiment the level of carboxyhemoglobin in the blood of the subjects had increased on the average from 0.7 to 2%. In 7 subjects out of 10, Yu. N. Tokarev found a drop in the voltage of the P, R and T spikes in the EKG (more often on the second standard lead) some narrowing of the QRS complex (in a number of cases, with speeding up of the heartbeat); the majority showed a decrease in the amount of oxygen consumed as well as in the coefficient of its utilization (B. S. Katkovskiy). In performing psychological tests (arithmetic calculation with switching) all the subjects made more mistakes than in an experiment with hermetic sealing.[17] At the same time, the ability to do the work with arithmetic tables remained practically the same (A. N. Kalinina). In biochemical studies, Z. I. Piliplyuk observed a normalizing effect of CO on the cholinesterase of blood serum, the activity of which dropped under the influence of hermetic sealing.

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[17] Yu. G. Nefedov and S. N. Zaloguyev (1967) suggest that the phenomena of CO autointoxication can be evaluated on the basis of an increased level of HbCO in the blood and a change in the CNS.

Thus, the experiments conducted supported the view that the concentration of carbon monoxide in the expired air is actually the "critical" index for its normality. At the same time, they showed that a CO concentration on /113
the order of 0.01 mg/liter can be viewed as the allowable limit for an 8-hour exposure. At more prolonged exposures its concentration in the air of hermetically sealed chambers must be less than 0.01 mg/liter. However, its concrete value for a period of exposure greater than 8 hours must be established in a special experiment of corresponding duration.

The above mentioned indices, of course, are not the sole criteria limiting the concentration of products of vital activity in the air of hermetically closed systems. In establishing the PLC of metabolic products that have an unpleasant odor it is also necessary to take into account the level of the threshold of olfactory sensitivity and in many cases also the level of the threshold of reflex activity of the substance. It is obviously particularly necessary to take these factors into account in calculating the PLC for a stay of many months in an artificial atmosphere in a hermetically sealed environment.

The values of the thresholds of olfactory sensitivity and reflex activity for some products of human vital activity are listed in Table 39.

In working out the PLC for harmful impurities in the artificial atmospheres of hermetically sealed environments it is possible in only a few cases to apply the PLC of these substances to the air medium of working environments or atmospheres of inhabited areas (Kustov, 1961) (Table 40).

The use of data on the PLC value for working environments and the atmospheres of inhabited areas in working out the PLC for hermetically sealed cabins of aircraft must be strictly limited. The PLC for working environments cannot be used to any significant extent for the following reasons.[18] These PLC's are calculated for an 8-hour exposure, 6 times a week for the period of

[18] For articles on the impossibility of extrapolating the PLC for working environments to the conditions of ecologically closed systems, see O. G. Gazenko, A. M. Genin (1967), G. Stokinger (1967) and A. Thomas and K. Beck, 1967.

a work shift. On the other hand, continuous exposure to poisons under the conditions of ecologically closed systems has completely different qualitative and quantitative characteristics. In addition, the PLC for working environments does not take into account the effect of a complex of factors of the external medium that are characteristic of the conditions of space flight (ionizing radiation, hypodynamia, weightlessness, presence of other harmful impurities, etc.). At the same time, however, in working out the PLC one must proceed on the basis of concrete conditions and take into account the effect of an entire complex of factors peculiar to the goal for which the PLC is being determined. Attempts at a mathematical method of extrapolating the PLC for working environments to the conditions of hermetically sealed cabins have been subjected to justifiable criticism (Gazenko and Genin, 1967). /114 Equally unjustified would be the application of PLC values for atmospheric pollution to hermetically sealed environments. These PLC's are calculated for long-term and continuous exposure for the period of a human lifetime. /115 These PLC's take into account the possibility of action of normalizing substances on the elderly, the young, and the sick (V. A. Ryazanov, 1954). However, like the PLC's for working environments, they do not take into account the possibility of exposure to additional factors that affect the conditions in hermetically sealed cabins. The PLC for harmful substances for working environments and the atmospheres of populated areas can find only limited use in the process of developing PLC's for hermetically sealed cabins. They can be used only as guidelines in selecting one concentration or another for experimental testing.

All of the above indicates the need for special derivation of the PLC's of harmful impurities for the artificial atmospheres of hermetically sealed environments, taking into account the principles set forth in this chapter.

TABLE 39. THRESHOLDS OF ACTIVITY OF CERTAIN PRODUCTS OF HUMAN VITAL ACTIVITY

Substance	Threshold of olfactory sensitivity, mg/m^3	Threshold of reflex activity		Source
		Value mg/m^3	Recorded reaction	
Ammonia	—	20	Cutaneous galvanic reaction	Alpatov, 1962
	—	40—50	EEG	»
	0,5	0,45	Sensitivity of eyes to light	Sayfutdinov, 1967
	»	0,35	EEG	
Acetone	1,1	0,55	Sensitivity of eyes to light	Feldman, 1962
	—	0,44	Electrocortical reflex	
	1,096	—	—	Tkach, 1967
	1,1	—	—	Pogosyan, 1967
	—	0,12	Cutaneous galvanic reflex	Piliplyuk, 1963
Methanol	4,3	3,3	Sensitivity of eyes to light	Chow Chen Tsi, 1961
	4,5	3,15	Ditto	Ubaydullayev, 1961
	—	1,17	EEG	
Phenol	3,5—4,0	—	—	Alekseyeva (cited in Beryushev, 1959)
	0,022	0,0155	Sensitivity of eyes to light	Mukhitov, 1963
	—	0,0156	Electrocortica reflex	
	0,022	—	—	Nogosyan, 1967
	0,017—0,022	—	—	Korneyev, 1967
Hydrogen sulfide	0,04	—	—	Loginova, 1955
	0,014	—	—	Baykov, 1964
	0,1	—	—	Sginev, 1960
Carbon monoxide	1,6—2	0,6	Sensitivity of eyes to light	Dubrovskaya, 1955
	—	0,6	Electrocortical reflex	Bushtuyeva, 1961
Sulfur anhydride	—	0,5%	EEG	Yeliseyeva, 1966
Skatole	1,2-unpleasant fecal odor	—	—	Dalla Velle, Dudley, 1939
Acetic acid	5,0	1,0	Cutaneous galvanic reflex, depression of alpha-rhythm on EEG	Mikhaylov, 1965

Commas indicate decimal points.

TABLE 40. PLC FOR WORKING ENVIRONMENTS AND ATMOSPHERES OF INHABITED AREAS

Substance	PLC* for working environments (8 hrs., 6 times a week), mg/m³	PLC** for populated areas (main daily value), mg/m³	Substance	PLC* for working environments (8 hrs., 6 times a week), mg/m³	PLC** for populated areas (main daily value), mg/m³
Ammonia	20,0	—	Carbon monoxide	20,0	1,0
Acetone	200,0	0,35	Hydrogen sulfide .	10,0	0,008
Dimethylamine . .	1,0	—	Methyl alcohol	50,0	0,5
Methyl mercaptan .	100,0 (USA)	—	Ethyl alcohol	1000,0	—
Methylamine . . .	5,0	—	Acetic acid . . .	5,0	—
			Phenol	5,0	0,01

*State Standard No. 245-63

**According to data of V. A. Ryazonov and M. S. Gol'dberg (1966).

Commas indicate decimal points.

CONCLUSION

The process of formation of new branches of science is going on at the present time. Toxicology of the atmospheres of hermetically sealed environments is one such new outgrowth of knowledge. Its development is linked to the need to solve complex problems dealing with the effect of chemical substances on the organism under the conditions of hermetically sealed cabins of aircraft, submarines or other hermetically sealed objects. These problems have received considerable treatment in the literature; pathways to their solution have been suggested (Lazarev, 1967, A Symposium on Toxicity in the Closed Ecological System, 1963; Proc. Conf. Atmospheric Contam. Confined Spaces, 1965). /116

Space toxicology is only one (but the most rapidly developing) twig on this branch. Problems of space toxicology, i.e., toxicology which satisfies the needs of designing, building and using spacecraft, have been discussed by us in a special paper (V. V. Kustov, L. A. Tiunov, 1968). Its problems and the methods of solving them have been formulated in many publications (cf. for example A. I. Burnazyan, Yu. G. Nefedov, V. V. Parin et al., 1967).

One of its chapters is the toxicology of gaseous products of human vital activity and their significance for the formation of artificial atmospheres for spacecraft cabins. One feature of this chapter is its close relationship to biochemistry, pathophysiology, sanitary chemistry, industrial toxicology, work hygiene and communal hygiene. Biochemical studies are required to investigate the mechanisms of formation of the products of human vital activity and to study the effect of various extremal factors on this process, features of nutrition, programs of work and rest, i.e., all that is imposed by the conditions of space flight. In addition, biochemical methods as well as pathophysiological and pathomorphological procedures are used to investigate the biological effect of gaseous products of vital activity and their compensation.

Sanitary-chemical studies with broad application of physical and chemical methods and methods of analytical chemistry are conducted to inter- /117

pret the composition of the elaborate complex of gaseous products excreted by man in order to study the kinetics of their excretion and to evaluate the influence of various factors of the external medium on their qualitative and quantitative composition. Methods of industrial toxicology, work hygiene and communal hygiene are used in working out the highest permissible concentrations.

In this paper we have studied successively the mechanisms of the formation of gaseous products of vital activity and the influence of various factors on this process; we have given qualitative and quantitative characteristics of the excretion of several gaseous products of vital activity that are excreted with the expired air, urine, feces and sweat of man. We have evaluated the contribution of this complex of substances to the formation of the gaseous medium; we have presented materials on the biological effect of the products of vital activity and the principles of their formation on the conditions of hermetically sealed environments. All of these materials are required for forming ecological closed systems of spacecraft cabins. As a matter of fact, the construction of life-support systems which guarantee maintenance of the parameters of the air medium at a certain level, requires data on quantitive characteristics and kinetics of excretion of gaseous substances that enter the atmosphere. These data, as well as the information on the levels of permissible concentration, are required for calculating the effectiveness of systems for purifying and regenerating the air.

Many problems of the toxicology of gaseous products of vital activity and their significance in the formation of artificial atmosphers deserve further study. They include, in particular, a broad study of the effect of extremal factors of space flight, work and rest programs, programs of nutrition and the formation and excretion of gaseous products of human vital activity. Considerable promise is offered by work on the further analysis of the chemical composition of gaseous products of human vital activity, the study of the kinetics of their excretion, further intensification of the study of the biological effect of the complex of metabolic excreta and finally, compensation for the conditions which are characteristic of hermetically sealed environments.

The present work is only an initial attempt at generalization in this area. The authors welcome any suggestions for its improvement.

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Translated for the National Aeronautics and Space Administration under contract No. NASw-2037 by Techtran Corporation, P. O. Box 729, Glen Burnie, Maryland, 21061.

NASA-Langley, 1971 — 4 F-634

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